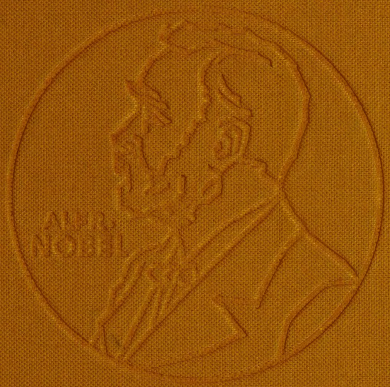

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Foreword

The Nobel Foundation has, by agreement, granted the Elsevier Publishing Company of Amsterdam the right to publish in English language the Nobel Lectures for 1901–1962. The lectures in the five Nobel Prize domains: Physics, Chemistry, Physiology or Medicine, Literature, and Peace will appear separately, according to the subject. The scientific lectures will each cover three volumes and those in literature and peace one volume each. Short biographical notes and the presentation speeches will also be included.

The Nobel Foundation has since 1901 each year published *Les Prix Nobel* which contains all Nobel Lectures of that year, always in the language in which they were given, as well as short biographies of the laureates. In addition an account is given of the prize-award ceremonies in Stockholm and in Oslo, including presentation addresses and after-dinner speeches, etc., thus covering the whole field of Nobel Prize events of one particular year.

In the Elsevier series the Nobel Lectures, presentation addresses, and biographies will now be more readily accessible to those who wish to follow the development in only certain of the Nobel subjects, as reflected in the prize awards during the years passed. For practical reasons English has been chosen as common language for this series.

It is the hope of the Nobel Foundation that the volumes to be published by Elsevier Publishing Company will supplement *Les Prix Nobel* and that together they will serve to spread knowledge of those landmarks on the road of human progress that have been honoured by Nobel Prizes.

Arne Tiselius – President, Nobel Foundation

Publisher's Note

During the realization of this work we have been confronted by many problems. In solving the great majority of them we have relied heavily upon assistance, both invited and spontaneously offered, from many quarters.

To some problems there was no solution to be found; in particular, it proved impossible to obtain, for some of the lectures, the original photographs or blocks used for the illustrations. As an inevitable consequence of this it was found necessary to reproduce these illustrations from a printed original; this naturally renders them less perfect than we would have wished. We thank those who were able to lend us original material.

The translations of those lectures which were delivered in a language other than English were prepared by the Babylon Translation Service, London. We are indebted to Mr. D. F. Styles and Mr. M. Fedorski (both of Manchester), and to the Babylon Translation Service, for research on, and preparation of, most of the biographies. Completion of these, and the addition of much detail, was achieved with the cooperation of many of the laureates themselves, their colleagues and assistants, and other individuals whose help was requested. To all of them we would like to offer our sincerest thanks in appreciation of their efforts.

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Chemistry 1942

Prize not awarded.

Chemistry 1943

GEORGE DE HEVESY

«for his work on the use of isotopes as tracers in the study of chemical processes»

Chemistry 1943

*In regard to de Hevesy's work, Professor A. Westgren, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences, made the following statement**

When, in 1913, de Hevesy was working with Rutherford in Manchester, this young scientist had been commissioned to isolate radium D from radioactive lead. His efforts were unsuccessful. It had in fact become apparent that radioactive radium D differed so little from inactive radium G, the last of the series of descendants of radium, that all attempts to isolate them from each other seemed destined to failure. The reason for this was at the same time discovered. Radium D and radium G are isotopes and constitute different species of lead. They differ in their atomic weight whilst their atoms have the same nuclear charge. The shells of their electrons, shells which determine their chemical properties, are therefore more or less identical.

Although unsuccessful, de Hevesy's efforts were not wasted. They gave him the idea for a new method of chemical research.

If it is impossible to isolate chemically a radioactive isotope from an element of which it is part, it must be possible to use this peculiarity to follow in its details the behaviour of this element during chemical reactions and physical processes of different kinds. The active atoms are recognized by their radiation and, being faithful companions of the inactive atoms of an element, they serve as markers for them. Since the intensity of radiation can be determined with such precision that imponderable quantities can be measured in this way, extremely small quantities of a marker of this kind are sufficient.

By using radium D as a marker, de Hevesy determined the solubility of highly insoluble lead compounds. He succeeded in determining exactly the quantity of lead sulphide or of lead chromate taken up under different conditions from solvents of different types. He studied the exchangeability of lead atoms into the dissolved substances and was able to confirm that it corresponded to the behaviour of the lead atoms as ions. The movements of the atoms in solid lead, i.e. the self-diffusion which occurs in this metal, would be determined; it had previously been impossible to measure this process. By

* Talk given on the radio on 10th December, 1944, with amendments and additions

precipitating thorium B, a very active isotope of lead, on the surface of a lead crystal and by following the reduction in radiation intensity brought about by the changes in place of the active atoms with the inactive lead atoms of the lower layer, and hence with the penetrations which took place in the crystal, he was able to measure the energy needed to liberate an atom from the crystallised part of the lead, in other words the dissociation energy of the crystal lattice. This energy was found to be of the same order of magnitude as the heat of vaporisation of lead. This latter research is particularly interesting from the physico-chemical point of view.

The new method has also enabled biological processes to be studied. Beans placed in solutions containing lead salts with a mixture of active lead atoms absorbed a part of these salts but the distribution of the metal was not the same in the root, the stem and the leaves. Most of the lead, which does not favour natural biological development but on the contrary acts as a poison, stays in the root. Relatively more lead was extracted from dilute than from more concentrated solutions. Absorption and elimination of lead, bismuth and thallium salts by animal organisms was studied in this way. A knowledge of the distribution of bismuth compounds introduced into an animal organism is valuable from the medical point of view, since some of these compounds, as we know, are used therapeutically.

So long as natural radioactive elements only were used as markers, use of the new method was inevitably very limited. In fact the method could be applied only in the case of heavy metals–lead, thorium, bismuth and thallium –and their compounds. The situation was to be very different when Frédéric and Irène Joliot-Curie, and Fermi succeeded in producing radioactive isotopes from any element by bombarding it with particles. This discovery was made some ten years ago and the study of chemical processes by means of radioactive markers has since then been carried to such a point that it is now widely used in laboratories throughout the world. De Hevesy has remained the prime mover in this new field of activity and much first-class and important research has been carried out by him and his co-workers.

Exceptionally valuable results have thus been obtained in biology. An isotope of radioactive phosphorus, which can be obtained by exposing sulphur to neutron radiation or ordinary phosphorus to radiation from nuclei of heavy hydrogen, has mostly been used. This radioactive phosphorus is sufficiently long-lasting for tests of this nature. It has a half-life of approximately 14.8 days. De Hevesy produced physiological solutions of sodium phosphate containing this marker and injected them into animals and hu-

mans. The distribution of the phosphorus was determined at certain intervals. A study of blood samples showed that the phosphorus thus introduced quickly left the blood. In human blood the radio-phosphorus content had fallen after only 2 hours to a mere 2% of its initial value. It diffuses into the extra-cellular body fluid and gradually changes places with the phosphorus atoms of the tissues, organs and skeleton. After some time it can even be found, though in very small quantities, in the enamel of the teeth. Exchanges small and slow as they may be, therefore occur between the outer hard parts of the teeth and the inner tissues of the bones and the lymph. Most of the phosphorus introduced, finds its way into the skeleton, muscles, liver and gastro-intestinal organs. Elimination of phosphorus from living organisms has also been studied by this method.

Phosphorus is an extremely important element in biological processes. The knowledge of its functions in living organisms which has been acquired thanks to the use of radioactive markers is therefore of the very greatest interest. De Hevesy succeeded in detecting where and at what speed the various organic compounds of phosphorus are able to form and the paths which they take in the animal organism. In order to form from a phosphate which has been injected into the blood they must first penetrate into the cells. Acid-soluble compounds of phosphorus form rapidly, whereas phosphatides closely related to fatty substances are slower-forming. These latter form mainly in the liver, whence they are carried by the blood plasma to the places where they will be consumed. De Hevesy showed that the phosphatides of the chicken embryo are produced in the embryo itself and that they cannot be extracted from the egg yolk.

De Hevesy also carried out several investigations with radioactive sodium and potassium. He studied how physiological saline containing radioactive sodium which was injected into a human subject first spread into the blood and then slowly penetrated into the cells; he also studied the manner in which it is excreted. After 24 hours the blood corpuscles had lost approximately half their sodium content.

In addition to the above-mentioned markers, several other active isotopes, such as magnesium, sulphur, calcium, chlorine, manganese, iron, copper and zinc, have been used for this type of research. In the case of the lighter elements it has also been possible to use inactive isotopes such as heavy hydrogen, with an atomic weight of 2, nitrogen, with an atomic weight of 15, and oxygen, with an atomic weight of 18. It is of course less easy to determine the content of an inactive than of an active marker, but this can be

done by determinations of density or mass-spectrographically. To determine the concentration of deuterium, or heavy hydrogen, which is twice as heavy as ordinary hydrogen, is a relatively easy matter. De Hevesy used deuterium as marker in many tests. He then noticed that a person who has drunk water containing heavy hydrogen excretes deuterium in the urine after only 26 minutes. Frogs and fishes swimming in water containing deuterium absorb it and, after about 4 hours, are in equilibrium with the medium as far as the deuterium is concerned. Heavy nitrogen and heavy oxygen have also been used in many investigations.

George de Hevesy

Some applications of isotopic indicators

Nobel Lecture, December 12, 1944

The method of isotopic indicators had its ultimate origin in the Institute of Physics at the University of Manchester, which then was under the inspiring leadership of that great physicist the late Lord (then Professor) Ernest Rutherford.

The cradle of radium is the Czecho-Slovakian town Joachimstal; it was from Joachimstal pitchblende ore that Professor and Madame Curie isolated that element. The Austrian Government, the owners of these mines, generously supplied Professor Rutherford not only with radium, but also with the by-products of radium production, equally important for the worker in the field of radioactivity. One of the most significant by-products is radium D, which has a half-life period of 20 years and is found associated with the very substantial amounts of lead present in pitchblende. The Austrian Government presented to Professor Rutherford several hundred kilograms of such «radio-lead». In view of its association with very large amounts of lead, which absorb the radiation emitted by radium D, this precious radioactive material nevertheless proved to be almost useless. When I met Professor Rutherford one day in 1911 in the basement of the laboratory where the radio-lead was stored, he addressed me in his friendly and informal way, saying: «My boy if you are worth your salt, you try to separate radium D from all that lead». In those days, I was an enthusiastic young man and, on immediately starting to attack the problem suggested to me, I felt quite convinced that I would succeed. However, although I made numerous attempts to separate radium D from lead and worked for almost two years at this task, I failed completely. In order to make the best of this depressing situation, I decided to use radium D as an indicator of lead, thus profiting from the inseparability of radium D from lead. Suppose that we dissolve 1 g of lead in the form of nitrate in water, add radium D of negligible weight showing a radioactivity of one million relative units (an electroscope being used to measure the activity), and proceed to carry out the most intricate operations with this «labelled» lead. If we then ascertain the presence of one radioactive unit in a fraction obtained in the course of these operations, we

must conclude that 1/1000 mg of the lead atoms present in the lead nitrate we started from, are now present in the fraction.

Radium D cannot be separated from lead, but it can easily be obtained in the pure form from lead-free radium salt samples or from radium emanation, since radium D is formed in the course of the disintegration of these radioactive bodies and can readily be separated from them. At that time, the Vienna Institute of Radium Research had more radium and radium emanation at its disposal than any other institution. This fact induced me, late in 1912, to start work at the Vienna Institute in collaboration with Dr. Paneth, assistant at that Institute, who himself had made very extensive and abortive trials to separate radium D from lead. The first application of labelled lead[1] was the determination of the solubility of some very slightly soluble lead compounds such as lead chromate. In these experiments, not radium D but another isotope of lead, thorium B, was applied as an indicator. Labelled lead chromate was obtained by adding a solution of 100,000 relative units of thorium B to lead nitrate containing 10 mg of lead and converting the nitrate thus labelled into chromate.

After the saturated solution of this compound had been held at the desired temperature in a thermostat for a sufficient time, its composition was ascertained by evaporating a few cubic centimetres to dryness and measuring the activity of the almost invisible residue in the electroscope. From the number of units of thorium B found, the amount of lead was calculated, one unit corresponding to 10^{-6} grams of lead; finally, the solubility of the lead chromate in moles per litre ($2 \cdot 10^{-7}$) was computed.

The radioactive method is extremely simple, having the advantage that the presence of foreign ions in no way interferes with the measurements. The method may be applied without difficulty, for example, in determining the solubility of lead sulphate in the presence of calcium sulphate.

Simultaneously with the said experiments, we used labelled lead and labelled bismuth (the radioactive bismuth isotope radium E can easily be obtained from radium, radium emanation, or radio-lead) in an investigation of the manner in which unweighable amounts of metals are precipitated during electrolysis[2]. The application of the well-known Nernst formula

$$e = \frac{RT}{n} \log_n \frac{c}{C}$$

was extended to concentrations of 10^{-8} N and even lower.

On the basis of Nernst's theory, we should expect an interchange to take

place between the metal of the electrodes and the ions in solution. The existence of such an interchange was demonstrated[3]. While, in the case of lead peroxide, the interchange was found to take place between the outermost layer of molecules of the geometrically calculated electrode surface, in the case of the lead electrodes numerous layers of molecules were found to participate in the interchange process. This finding is to be interpreted as a result of local currents due to variations in the structure of the metallic surface. An interchange between atoms of a lead foil and the lead ions present in a solution was found to occur very rapidly, while the lead ions adsorbed on colloidal lead particles were found to interchange at a slow rate only[4].

In contrast to metallic surfaces, Paneth[5] found that in the case of salt crystal surfaces the interchange was restricted to the uppermost molecular layer of the crystal. On this observation he based an important method for the determination of the surface areas of crystalline powders[6].

When lead sulphate is shaken with its saturated solutions, a constant kinetic exchange occurs between the molecules of lead sulphate in the solution and those on the surface of the solid. If the solution contains marked molecules, after equilibrium has been attained the numerical ratio of marked molecules on the surface to those in solution is identical to that of total molecules on the surface to total molecules in the solution. As the distribution of the labelled molecules is determined by means of radioactive measurements, and as the lead sulphate content of the saturated solution is evaluated by the usual methods of analytical chemistry, the amount of lead sulphate present in the uppermost molecular layer can be computed. When the weight is known of a unimolecular layer of lead sulphate of 1 cm^2 area, the surface of the crystal powder can be calculated from the above data.

Among the numerous applications of radioactive indicators by Paneth I wish to emphasize the importance of his discovery of the existence of bismuth hydride[7] and lead hydride[8]. After he gained experience regarding the best method of preparation and the stability of radioactive bismuth hydride and lead hydride, he succeeded in preparing these compounds from inactive bismuth and inactive lead, respectively.

Self-diffusion

The conception of the diffusion of a substance into itself, self-diffusion, was introduced by Maxwell. No further use was made of this concept until fifty

years later, when the method of radioactive (isotopic) indicators was introduced. The possibility of measuring self-diffusion by following the rate of penetration of the lead isotopes ThB or RaD into lead soon suggested the measurement of the self-diffusion in liquid and solid lead, using ThB or RaD as indicators. The measurements of the self-diffusion coefficient in liquid lead[9] gave the result anticipated from the known diffusion rates of lead in mercury and other related elements. The diffusion rate in liquids is primarily determined by the radius of the diffusing particle and the viscosity of the liquid: thus, the replacement of a diffusing metal present in small concentration by another related metal will not appreciably influence the rate of diffusion. A very different behaviour was revealed, however, when the self-diffusion in solid lead[10] was measured, using RaD as indicator. In the first experiments carried out in collaboration with Gróh, we soldered a piece of radio-lead to the bottom of a rod of ordinary lead, whereafter the system was kept at 280° for 140 days. After the lapse of that time, we cut the system into four equal parts, rolled the four lead pieces into thin plates, and placed them in an electroscope. No diffusion of the radio-lead into the ordinary lead could be ascertained, showing that the self-diffusion rate in lead must be at least several hundred times smaller than that of gold in lead, as determined by Roberts-Austin.

This result necessitated the introduction of special methods of great sensitivity for measuring diffusion. Since the rate of diffusion is inversely proportional to the square of the thickness of the layer, we worked out methods for the measurement of the penetration of radioactive lead into ordinary lead layers to a depth of only a few microns. The α-particles emitted by ThB (more correctly, by its disintegration products, ThC and ThC′, which, however, attain radioactive equilibrium with the former within a few hours) produce scintillations on a zinc sulphide screen, the number of which is ascertained. The infinitesimal layer of ThB, which is in intimate contact with a lead foil placed below it, is then heated for a few hours to, say, 200°. If a diffusion of the ThB atoms into the lead foil takes place, the count of the scintillations will give a smaller value after the experiment than before. The range of the α-particles in lead being only about 30 μ, a shift of a small percentage of the ThB atoms to depths less than 30 μ will suffice to reduce the counts of scintillations to a noticeable extent. This method, worked out in collaboration with Mrs. Obrusheva[11], was later replaced by a more sensitive and exact procedure applied in diffusion measurements in solid metals, and also salts, in collaboration with Seith[12]. ThB was condensed on a foil

or a single crystal of the metal, and the ionisation produced by radiation emitted by the radioactive body was measured. A slight diffusion of the ThB into the lead after heating sufficed to diminish the ionising effect registered by an electrometer. Instead of the ionisation produced by the α-rays, the ionisation produced by recoil particles accompanying the emission of α-rays was measured in some cases. The range of the recoil particles in lead being only about 10^{-5} cm, these measurements made possible the determination of diffusion coefficients as small as 10^{-12} cm^2 per day.

Our measurements led to the result that, while the diffusion coefficient of gold in lead is found to be $5 \cdot 10^{-3}$ cm^2 day^{-1} at 165°, the coefficient of self-diffusion in lead at the same temperature is only 10^{-6} cm^2 day^{-1}, the difference rapidly increasing with decreasing temperature. The change of the value of the coefficient of self-diffusion, D, in lead foils and single crystals is represented by the equation

$$D = 5.76 \cdot 10^5 \, e^{-27900/RT} \text{ cm}^2 \text{ day}^{-1}$$

Making use of this formula, we can show that, at room temperature, the atoms will change their places in a piece of lead on the average only once a day.

From the change of the coefficient of self-diffusion with temperature, the heat of activation of the diffusion process, the heat of loosening of the lead lattice, can be calculated. The value obtained and, for purposes of comparison, other thermal data are given in Table 1.

Table 1. Thermal data for solid lead

	kcal per g atom
Heat of melting	1.1
Energy content at the melting point	3.5
Heat of lattice-loosening	27.9
Heat of evaporation	36.2

Roberts-Austin measured the diffusion rate of gold in solid lead. His measurements gave the first quantitative determination of diffusion rates in solids. The high values he obtained, shown in Fig. 1[13], led his contemporaries to consider diffusion in solid metals a comparatively rapid process. The introduction of the conception of self-diffusion and the subsequent development led to a very different view and also to the elucidation of the remark-

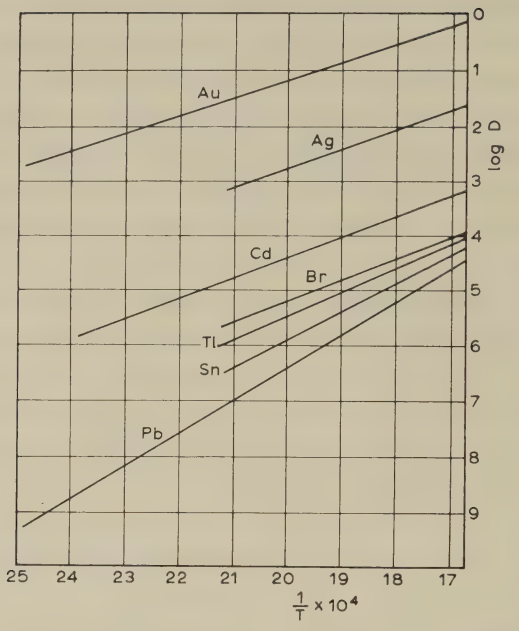

Fig. 1. Diffusion rates of metals in solid lead.

able nature of the gold-lead system investigated by that pioneer metallurgist.

The methods outlined above were also applied to determine the self-diffusion rate of Pb^{++} in solid lead chloride and lead iodide[12]. The variation of the self-diffusion rates with temperature can be expressed by the equations

$$D = 1.06 \cdot 10^7 \, e^{-38120/RT} \text{ and } D = 3.43 \cdot 10^4 \, e^{-30000/RT}$$

respectively. As first shown by Nernst, the ionic mobilities in an electrolyte solution, and hence the conductivity of the solution, can be calculated if the diffusion rates of the ions are known. We can apply the same ideas to solid electrolytes[14] and calculate, for example, the diffusion rate of Pb^{++} from the conductivity of lead chloride, on the assumption that the electrolytic conductivity of the salt is due solely to the transference of charges by the Pb^{++}. The diffusion rate of Pb^{++} thus calculated is, however, many thousand times larger than the value found experimentally, showing that the chloride ions are almost exclusively responsible for the conduction of electricity in solid lead chloride. The above data permit the calculation of the transport number of Pb^{++} in solid lead chloride. Not far from the melting

point, at 484°, the transport number of Pb^{++} is found[15] to be 10^{-3}, at 273° 10^{-5}, and at 90° only 10^{-10}. By no other method can such small transport numbers be determined in any electrolyte.

In the case of lead iodide, the diffusion rate of Pb^{++} calculated from conductivity data, under the assumption that the whole conduction is due to Pb^{++}, is in good agreement with the measurement of the self-diffusion rate of Pb^{++}. This shows that, in contrast to the case of $PbCl_2$, the conductivity in PbI_2 at high temperatures is due almost exclusively to the transference of charges by Pb^{++}. With decreasing temperature the rôle of Pb^{++} decreases and the transport number of I^- increases accordingly. At 260°, only 40% of the conductivity is due to Pb^{++} and 60 percent to the I^-; at 155° the share of the former is only 0.4 percent. In the case of lead iodide, Tubandt's beautiful method for the determination of transport numbers could also be applied. The values obtained agreed well with those found by the measurement of self-diffusion.

Formerly, the self-diffusion rates of only lead, bismuth and a few other elements could be determined. These elements have natural radioactive isotopes. The discovery of artificial radioactivity greatly enlarged the possibilities for the determination of self-diffusion rates. By making use of the radioactive bromine isotope, we can determine the self-diffusion rate of Br^- in AgBr just as we determined that of Pb^{++} in $PbCl_2$. Working at the Institute for Theoretical Physics at the University of Copenhagen, H. A. C. McKay determined the self-diffusion in gold. By the action of neutrons on gold a radioactive gold isotope, having the atomic number 198, can be produced. Neutrons having an energy of about 4 V are strongly absorbed in gold. A thin gold sheet exposed to the action of such neutrons will be more strongly activated on the side first struck by the neutron beam than on the opposite side. When the activated film is heated, the difference in the concentrations of the active gold atoms will decrease and, from the decrease of the activity difference shown by the two faces of the foil, the rate of self-diffusion in gold can be calculated[16].

Svante Arrhenius' theory of electrolytic dissociation

If we dissolve sodium chloride and the equivalent amount of sodium bromide in water and then separate the two salts by crystallisation, it would have been expected in the time prior to Arrhenius that the chloride ions

would retain their original partners, the same applying to the bromide ions. According to Arrhenius, however, each chloride ion has the same chance of associating with a sodium atom originally bound to chlorine as with one initially associated with bromine. The correctness of the much debated views of Arrhenius was shown in different ways; the most direct proof, however, was provided through the application of isotopic indicators[17]. When equivalent amounts of $PbCl_2$ and labelled $Pb(NO_3)_2$ (or *vice versa*) were dissolved and the two compounds were separated by crystallisation, the labelled lead ions were found to be equally distributed between chloride and nitrate ions.

Very different results were obtained in all cases in which the lead atom was joined to carbon. Between lead chloride and lead tetraphenyl in pyridine, between lead acetate and lead tetraphenyl in amyl alcohol, and between lead nitrate and diphenyl lead nitrate in aqueous ethyl alcohol, no change in the places of lead atoms could be detected, although in every combination investigated one of the molecular types was capable of electrolytic dissociation.

The lack of interchange of atoms present in organic binding (hydrogen atoms bound to oxygen or nitrogen being an exception, as shown by Bonhoeffer[81]), such as that of carbon atoms in glycogen or phosphorus atoms in lecithin with other carbon and phosphorus atoms respectively, was found to be of great significance for the application of isotopic indicators in biochemical research. Owing to the absence of such an interchange, the presence of labelled carbon atoms in glycogen molecules, or of labelled phosphorus atoms in lecithin molecules, extracted from the organs, proved that a synthesis of these molecules took place after the labelled atoms were administered. This principle enables us to distinguish between «old» and «new» molecules and to determine the rates at which molecules of the different compounds are built up and carried to the different organs.

A prompt interchange of the electrical charges between Pb^{++} and Pb^{++++} ions was found to take place in experiments where plumbous acetate and labelled plumbic acetate (or *vice versa*) were dissolved in glacial acetic acid and then separated by crystallisation[18]. The same holds for Tl^{+} and Tl^{+++} ions[19]. An interchange of lead atoms takes place between fused lead and fused lead chloride, lead oxide or lead sulphide[20].

After artificially radioactive isotopes became available as indicators, interchange processes were studied in numerous cases. A rapid interchange of charges was found to take place between Fe^{++} and Fe^{+++}, Cu^{+} and Cu^{++}, etc.[21].

Analytical chemistry

Analytical chemistry proved to be a fruitful field for the application of isotopic indicators. A knowledge of the total lead content of the earth's crust, for example, is of considerable chemical interest. In view of the small lead content of the average rock sample, the quantitative determination of its lead content involves some difficulties. These have been eliminated by making use of an isotopic indicator[22]. An amount of radium D, known in relative radioactive units, is added to the solution of the rock sample; the radium D is then recovered by electrolysis as peroxide. If 100 per cent of the added radium D is recovered, we may expect 100 percent of the lead present in the sample to have been recovered as well. If only 50 percent is recovered, for example, we have to multiply the amount of lead recovered by 2 in order to arrive at a correct analytical figure. The indicator method thus allows a correction for the shortcomings of the analysis. Such corrected analytical figures are seen in Table 2.

Instead of adding radium D to the solution to be analysed, we may add lead labelled by the presence of some radium D, for example 100 mg of lead having an activity of 1,000 units. If we subsequently isolate 10 mg of lead from the solution, this lead should show an activity of 100 units, under the assumption that the original sample does not contain lead. If the activity of the isolated 10 mg of lead is, for example, found to be 83 only, we have to conclude that the sample contains lead amounting to 20 mg.

In recent years, isotopic indicators have found an extended application in

Table 2. Lead content of igneous rocks

Rock types	*g lead per g rock precipitated by electrolysis*	*Percentage RaD recovered by electrolysis*	*Corrected value of g lead per g rock*
Gabbros and related types (composite of 67 samples)	$4 \cdot 10^{-6}$	80	$5 \cdot 10^{-6}$
Essexites and related types (composite of 40 samples)	$7 \cdot 10^{-6}$	80	$10 \cdot 10^{-6}$
Soda-granites and soda-syenites (composite of 26 samples)	$9 \cdot 10^{-6}$	73	$11 \cdot 10^{-6}$
Granite rocks (composite of 58 samples)	$18 \cdot 10^{-6}$	53	$30 \cdot 10^{-6}$
Basalt (Giant Causeway)	$4 \cdot 10^{-6}$	100	$4 \cdot 10^{-6}$

biochemical analyses. Schoenheimer and his colleagues[82] determined the leucine content in the protein of the rat by adding to the hydrolysate a known amount of leucine containing heavy nitrogen. This tracer was also used[83] in the investigation of the occurrence of the amino acids of the *d* series in cancer proteins; while Chargaff, Ziff and Rittenberg[25] used bases containing a known amount of ^{15}N in the analysis of the nitrogenous constituents of tissue phosphatides. Amino acids containing deuterium as indicator were used by Ussing[23] and the same tracer was applied by Rittenberg and Foster[24] in their determination of the palmitic acid content of rats' fat.

Early biological application

In contradistinction to the animal body, the uptake of mineral constituents by the plant is not followed by a loss of such constituents, and it was formerly considered that the ions taken up by the roots of the plant did not migrate in the opposite direction at all. The application of isotopic indicators, however, has shown that this is not the case. Ions taken up by the plant can be removed by an exchange process under the action of other ions present in the soil or in the nutrient solution. It was already found in 1923 that minute amounts of lead, labelled by the admixture of the lead isotope thorium B, when taken up by the roots of *Vicia faba*, could to a large extent be removed by an excess of non-labelled lead added to the nutrient solution[26]. Most other ions were found to be much less effective in removing the labelled lead ions from the plant.

In recent years, the behaviour of essential constituents of plants has been investigated, making use of artificial, radioactive ions as indicators; similar results were obtained. Mullins and Brooks[27] placed cells of *Nitella coronata* first in a solution containing radioactive potassium and later in solutions of different chlorides. Sodium and lithium were found to be much less effective in removing labelled potassium than potassium itself, whereas rubidium was more effective. Jenny and Overstreet[28] and Broyer and Overstreet[29] found that ionic exchange could take place during periods of, and under conditions favourable for, active solute uptake. It was also observed[30] that for each six phosphate ions taken up by the roots of growing wheat seedlings, one phosphate ion migrated from the roots into the nutritive solution.

Early in the twentieth century, the application of bismuth compounds in syphilis therapy came to the fore. This induced Christiansen, Lomholt and

de Hevesy[31] to investigate the absorption, circulation and excretion of labelled bismuth preparations. Lomholt[32] succeeded in showing that, of all the preparations investigated, bismuth hydroxide suspended in oil was most suitable for therapeutic application.

Successes achieved by Blair-Bell in cancer therapy, using lead compounds, induced the investigation of the partition of labelled lead compounds between normal and tumorous tissue[33]. Though this work gave a negative result, it nevertheless proved to be of great importance in the future development of isotopic indicators. It was in the course of these investigations that Schoenheimer became familiar with the method of isotopic indicators, which he applied several years later with such great success in the study of fat and protein metabolism and of numerous related problems. Never were more beautiful investigations carried out with isotopic indicators than those of the late Professor Schoenheimer, whose untimely and tragic death is much to be deplored. The discussion of the numerous important results obtained by Schoenheimer and Rittenberg and their collaborators[34] lies, however, beyond the scope of this lecture.

Heavy water

In 1931, Urey discovered deuterium, an isotope of hydrogen[35]. This important discovery made possible the labelling of hydrogen. Deuterium is not an ideal indicator, its properties differing appreciably from those of hydrogen. The latter has a unique position: it is the sole element met with, though only transitorily, as a naked nucleus in chemical reactions. Chemical forces do not suffice to remove all electrons from any other element. Differences in the structure of the nucleus will therefore make themselves more noticeable in the chemical behaviour of hydrogen isotopes than in the case of any other element. Furthermore, the difference between the mass of the hydrogen atom and that of the deuterium atom amounts to as much as 100 percent, while, for example, the corresponding difference between a ^{31}P and a ^{32}P atom is only 3 percent. The very small difference in the chemical properties of ^{31}P and ^{32}P remains at the present time within the errors of our experiments, whereas between hydrogen and deuterium the difference is quite appreciable. The same applies to H_2O and D_2O. Dilute «heavy» water, however, contains mostly DOH molecules which exhibit in their chemical behaviour a very great resemblance to HOH.

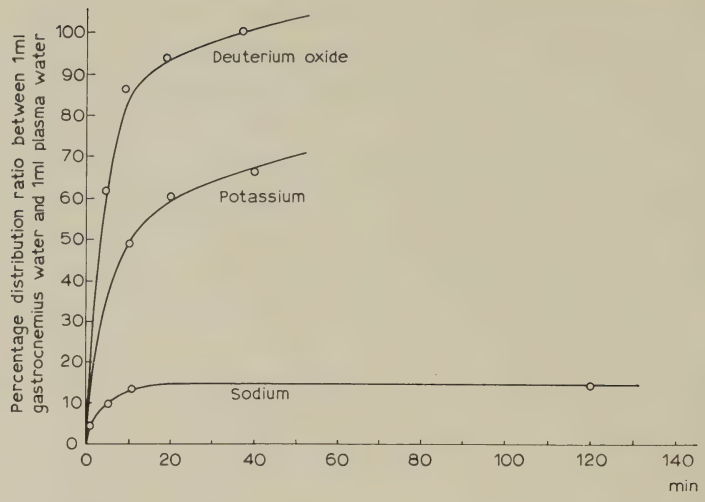

Fig. 2. Percentage distribution ratio of labelled sodium, potassium and deuterium oxide between plasma water and muscle water of equal weight.

In the study of the circulation of water in the organism, dilute heavy water can therefore safely be used as an indicator. In the determination of the life period of water molecules in the human organism, water containing $\frac{1}{2}$ per cent heavy water was used[36]. While a small percentage (0.1) of the water was found to be excreted in so short a time as 26 minutes, the average life of the water molecules in the organism was found to be 13.5 days. In the excreted water, molecules were thus found which were taken in both a few minutes and several months before the investigation. By extrapolation, however, we arrive at the result that, though the number of water molecules present in an adult organism amounts to as much as ca. 10^{27}, the adult organism no longer contains a single water molecule taken up at birth.

The rate of admixture of administered water with the water present in the body was investigated in experiments on rabbits[37] and guinea pigs[38]. While the water reaching the circulation was found to enter into exchange equilibrium with the extracellular water (about $\frac{1}{4}$ of the weight of the rabbit) in the course of a few minutes, the penetration of the water molecules into the cells took some time. As is seen in Fig. 2, about 30 min passes before the exchange equilibrium is reached between the water administered and extracellular and intracellular water present in the muscles of the rabbit. In the guinea pig, 73 per cent of the water in the blood is exchanged for extracellular water every minute.

Time does not permit me to discuss the extended application of heavy hydrogen, heavy carbon and heavy nitrogen, and to treat the numerous important results obtained by the use of these isotopes as indicators.

Application of artificially radioactive isotopes

During the lengthy operations preceding the early experiments of self-diffusion in lead, we often discussed the great progress which might be expected if radioactive indicators of the common elements were made available to chemical and biological research. This wish, which seemed utopian in those remote days, was fulfilled by Frédéric Joliot and Irène Joliot-Curie's[39] fundamental discovery of artificial radioactivity, followed by Fermi's[39] work leading to the discovery of many more artificially radioactive isotopes. Soon after the announcement of these discoveries, we prepared the radioactive phosphorus isotope ^{32}P by neutron bombardment of carbon disulphide and used this isotope in collaboration with Chiewitz[40] in the study of phosphorus metabolism. In these experiments, 10 litres of carbon disulphide were used to absorb most of the neutrons emitted by a mixture of radium and beryllium kindly put at our disposal by Professor Niels Bohr. The ^{32}P formed was extracted by treatment with diluted nitric acid or with water, the carbon disulphide being immediately available after the extraction for further neutron-irradiation.

A few other radioactive isotopes, such as the radio-halogens can also be prepared by similar simple and convenient procedures. This is, however, not the case with the majority of radioactive isotopes. These were prepared in amounts sufficient to be utilized in indicator work only after the invention of the cyclotron[41]. Lawrence's highly significant invention also made available radio-phosphorus preparations of very much greater activity than could be obtained from neutron-sources containing as much as several grams of radium. The number of neutrons produced by the Berkeley cyclotron was stated by Birge[41] in 1939 to correspond to the ionisation produced by 100 kg of radium; since that date, a still more powerful cyclotron has been brought into use. In our later investigations, radio-phosphorus generously put at our disposal by Professors Niels Bohr, Lawrence and Siegbahn was used.

The preparations of radioactive isotopes of numerous elements prepared in the Radiation Laboratory at Berkeley and in other laboratories found an extended application as indicators. Radioactive iron prepared at Berkeley, for

example, was used by Hahn, Whipple and their colleagues[42] in extended studies of iron metabolism.

The application of cyclotron-prepared radio-carbon[43] revolutionized our views of the fundamental process of photosynthesis.

Radio-iodine[44] found an extended application in the study of the formation of thyroxine and diiodotyrosine; it led, *inter alia*, to the important finding that some thyroxine is formed in the organism even after total extirpation of the thyroidea[45].

Radio-phosphorus found, however[46], the most extensive application. This was due not only to the convenient mode of production and period of decay of this material, together with the low absorbability of the rays emitted by it, but mainly to the important part which phosphorus plays in a very great number of metabolic processes. These include skeleton formation, metabolism of carbohydrates and fats, cell division, and many other processes. The discussion of the rôle of phosphorus in metabolic processes is therefore well suited to demonstrate different applications of isotopic indicators in biological research. We shall therefore now describe some applications of radio-phosphorus. These examples represent only a small percentage of the investigations in which radio-phosphorus has been used as an indicator; many of the results to be discussed were obtained in Copenhagen.

Radio-phosphorus

Owing to the great sensitivity of the Geiger-Müller counter, which registers ^{32}P with an activity of only 10^{-6} microcurie, some of the radio-phosphorus administered can soon be located in all organs. Table 3 shows the distribution of ^{32}P in the organs of the rat 4 hours after subcutaneous injection of labelled sodium phosphate[47].

While 4 hours after the administration most ^{32}P is found in the skeleton, muscles, liver and the digestive tract, with increasing time more and more ^{32}P becomes incorporated with the skeleton; 98 days after the start of the experiment, 92 percent of all ^{32}P present in the rat, which corresponds to about one half of the total amount administered, is found in the skeleton. This result may be seen in Table 4. Most phosphorus taken up with the food, in so far as it is not excreted, ultimately finds its way into the skeleton, where it replaces « old » phosphorus which interchanges with the phosphorus present in other organs or is excreted.

Table 3. Distribution of ^{32}P between different organs in a rat, 4 hours after subcutaneous injection of labelled phosphate (Weight of the rat: 188 g)

Organ	*Percent ^{32}P present*	*Specific activity*
Bones	22.6	0.020
Muscles	18.7	0.191
Liver	17.6	0.475
Digestive tract	15.9	0.365
Skin	11.1	0.192
Lungs and heart	6.3	0.317
Blood	2.5	0.558
Kidneys	2.4	0.370
Spleen	1.3	0.256
Brain	0.02	0.032

Table 4. Percentage total ^{32}P found in some organs of rats

Organ	*Time after distribution of ^{32}P*						
	Hours		*Days*				
	1/2	*4*	*10*	*20*	*30*	*50*	*98*
Muscles	18.3	19.4	25.8	28.8	25.2	12.1	3.6
Total skeleton	19.1	23.4	43.1	43.1	51.8	76.5	92.0

From these results, however, no conclusions can be drawn concerning the extent of renewal of the skeleton, as the labelled phosphorus, i.e. phosphorus administered throughout the experiment, may be incorporated wholly or principally in the upper molecular layers of the apatite-like crystals which form the mineral constituents of the skeleton. We can determine the extent of renewal of the bone mineral phosphorus by comparing the ^{32}P content, i.e. the radioactivity of 1 mg bone mineral P, with the radioactivity of 1 mg free plasma P. Were the bone phosphorus entirely renewed in the course of the experiment, the ^{32}P would be distributed equally between the free P atoms of the apatite-like bone crystallites and the free P atoms of the plasma, the latter being the direct or indirect source of the bone phosphorus. If only 1 per cent of the bone apatite P were renewed in the course of the experiment, the specific activity of the bone apatite P would be only 1/100 of that of the free plasma P.

The determination of the degree of renewal based on the said administration is made difficult by the fact that the specific activity of the free plasma

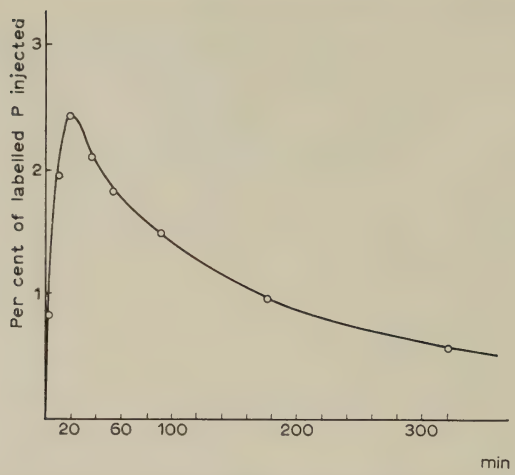

Fig. 3. Change in the specific activity of the plasma inorganic P after subcutaneous injection of labelled phosphate into a rabbit.

phosphorus varies throughout the experiment. After administration by the mouth or by subcutaneous injection, the specific activity first increases and subsequently decreases with time, while after administration by intravenous injection it first decreases very rapidly, and later at a moderate rate, as seen in Figs. 3 and 4[48]. The determination of the extent of renewal of the mineral P in the skeleton is much facilitated by keeping the free plasma P activity at a constant or almost constant level. This can be attained by repeated injections of varying amounts of labelled phosphate throughout the experiment. Making use of this technique, the data given in Table 5 were obtained for

Table 5. Extent of renewal of the mineral constituents of the bone in the course of 50 days

	Percentage renewal
Femur epiphysis mineral P	29.7
Femur diaphysis mineral P	6.7
Tibia epiphysis mineral P	28.6
Tibia diaphysis mineral P	7.6
Costa mineral P	27.5
Femur phosphatide P	100

the extent of renewal of the different parts of the skeleton of the adult rabbit in the course of 50 days[49]. It appeared that 72 per cent of the epiphysis and 93 percent of the diaphysis remained unchanged after this period, while 29

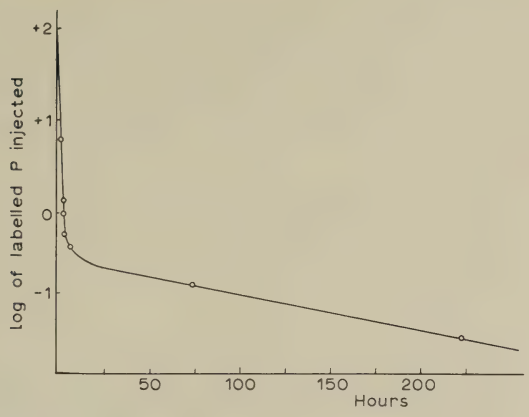

Fig. 4. Change with time in the logarithm of the labelled P content of the plasma after intravenous injection of labelled phosphate into a rabbit.

and 7 percent respectively were renewed not once, but, at least to some extent, repeatedly.

The restricted extent of renewal of the skeleton is due to the fact that while the P atoms of the uppermost molecular layer of the bone apatite crystals can promptly interchange with the free P atoms of the plasma (actually not the P atoms, but the phosphate ions interchange), a renewal of the main part of the apatite P can take place only when the crystal is dissolved and when crystals are formed from the plasma; from a labelled plasma, labelled crystals are formed. This « biological » recrystallization of the skeleton crystallites is a slow process. Moreover, we have to consider that if only the outer part of the crystal is renewed, this process can often be repeated without affecting deeper molecular layers of the crystal.

No data are available concerning the extent of renewal of the human skeleton; the relative rates of renewal of different parts of the skeleton were, however, determined by Erf[50].

The problem as to whether and to what extent the P atoms of the dental enamel are renewed has been a subject of extensive investigations[51] which led to the result that, though some ^{32}P is found to be present in the enamel after administration of labelled phosphate, the extent of renewal of the enamel phosphate is almost negligible. Regarding the extent of replacement of the phosphorus present in the constituents of the dentine, about one millionth part of the food phosphorus was found to be located in the mineral constituents of the dentine of each tooth.

The bone tissue growing in a labelled organism is bound to become labelled. Of the labelled phosphate administered by mouth, after the lapse of 3 days, 2 percent was found to be present in the rapidly growing incisors of the rat[52]. As seen in Fig. 5, these phosphate ions are mostly found in the

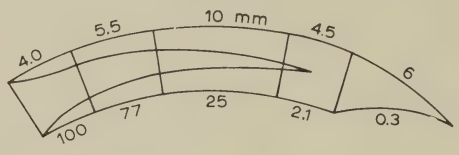

Fig. 5. Distribution of labelled phosphorus in the incisor of a rat killed 3 days after the administration of the phosphorus. The figures below give the relative amounts of labelled phosphorus present in 1 mg of fresh tissue in the section in question. The figures above give the length of the section in mm.

incisal part of the incisor, though a minor part are located at the apical end remote from the pulpa.

Permeability investigations

The above-mentioned rapid decrease in the plasma activity following intravenous administration of ^{32}P is to a large extent due to the interchange of plasma phosphate with the phosphate of the extracellular fluid. From this fact it follows that the capillary wall is readily permeable to phosphate; similar results were obtained for the other labelled ions investigated. Sodium ions, which are mainly confined to the extracellular space, enter into exchange equilibrium with the plasma sodium within 20 minutes[53,84,85]. This may clearly be seen from Fig. 6[53]. For potassium and for phosphorus, elements mainly located in the tissue cells, a longer time is required for the attainment of such equilibrium[54]. The low rate at which exchange equilibrium between the cellular and extracellular phosphorus is reached in the animal organism is mainly due to a very low rate of renewal of large parts of the skeleton.

As seen from the above examples, the method of isotopic indicators can be utilized with advantage in permeability investigations. It is with the aid of isotopic indicators that we best can measure the permeability of phase boundaries, since other methods do not indicate solely the resistance of the phase boundary to the penetration of ions, but a more complex phenomenon. Prior to the application of isotopic indicators, the high potassium content of the

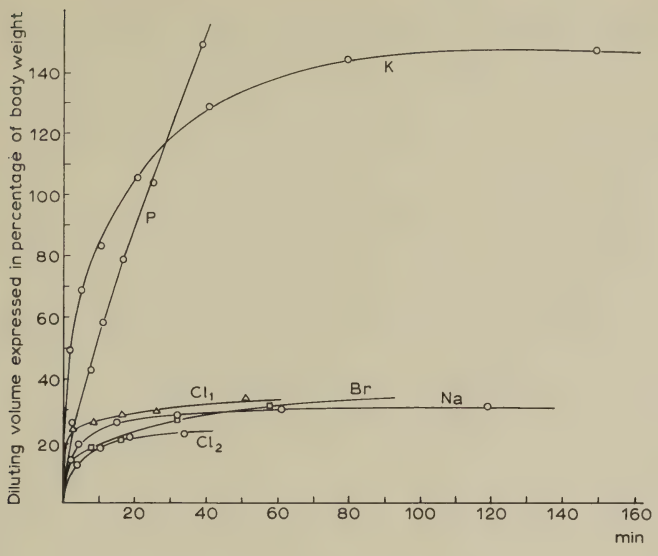

Fig. 6. Rate of disappearance of various labelled ions from the plasma.

erythrocytes of most species and their low sodium content were interpreted as being due to the impermeability of the erythrocyte membrane to potassium and sodium ions. The application of isotopic indicators, however, has disclosed the fact that potassium ions in the erythrocyte interchange quite easily[55] with those present in the plasma, and the same applies to the sodium ions. The high concentration of potassium and low concentration of sodium found in the erythrocytes of most animals can thus not be explained as being due to an impermeability of the corpuscle membrane to these ions.

Not only the resistance of phase boundaries to labelled ions, but also that to molecules of different kinds, can be measured with the aid of isotopic indicators. The rates of interchange of phosphatides present in the plasma and in different organs were determined in the following way[56]. Labelled phosphate was administered to a rabbit. After the lapse of 2 days, when the plasma contained an appreciable amount of labelled phosphatides, part of the plasma of another rabbit (rabbit II) was replaced by the labelled plasma. On following the decrease with time in the activity of the phosphatides extracted from the plasma of rabbit II, it was found that half of the plasma phosphatide molecules had interchanged in the course of few hours with phosphatide molecules present in the organs of the rabbit. An investigation of the activity of the phosphatides isolated from the organs led to the result that a very

substantial part of the labelled phosphatide molecules injected was found in the liver.

Rate of formation

The rate of formation of labelled organic phosphorus compounds differs much for various compounds and varies greatly with the organ in which they are located. The labile P of adenosine triphosphate, for example, is renewed at a very remarkable rate[57,86], the second P atom being renewed somewhat more slowly than the terminal atom[58]. Hexose monophosphate, present in the red corpuscles, was found to be largely renewed within a few minutes[57]. The formation of labelled phosphatides takes place in the liver and in the intestinal mucosa of the rabbit at a much more rapid rate than in the brain and more quickly than in any other organ[59]. Desoxyribose nucleic acid, on the other hand, shows a behaviour opposite to that of the phosphatides. Its extent of renewal in the liver of adult rats is very low[60,61,87], amounting to only about 0.1 percent in the course of 2 hours. In the spleen and in the intestinal mucosa, the renewal of the desoxyribose nucleic acid is 20 and 30 times, respectively, more rapid than in the liver. High figures for the rate of formation were found in rapidly growing tissue.

X-rays were found to obstruct the formation of labelled desoxyribose nucleic acid molecules[61]. Following irradiation with 300 r or more both in the sarcoma and in the organs of the rat formation figures were obtained amounting only to about half of the value observed in the controls. In the study of the reduction in labelled nucleic acid formation under the action of X-rays, a new line of attack was opened in the study of the action of such radiation on cell division.

We calculate the extent of renewal of the compound in question, for example creatine phosphoric acid, by comparing the specific activity of the creatine phosphorus at the end of the experiment with the average specific activity of the free phosphorus present in the tissue cells during the experiment. This calculation is based on the assumption that the labelled free phosphate, or the phosphate of a donor whose P enters rapidly into exchange equilibrium with the free P present in the cells, is incorporated in the creatine phosphate molecule present in the cells of the organ investigated. In the case of phosphatides, the possibility cannot be entirely excluded[45,46] that a precursor of the phosphatides containing labelled phosphate is transferred from another organ into the organ investigated. In such a case, the calcula-

tion of the extent of the renewal of the phosphatide molecules would necessitate knowledge of the specific activity of the precursor P. The renewal figures obtained must therefore be interpreted with caution. Another more pertinent reason for the cautious interpretation of the results obtained is that the molecules of some organic compounds may possibly be built up within the phase boundary, where the specific activity of the free P may appreciably differ from that of the intracellular free P.

While we measure the rate of renewal of phosphatide molecules with respect to their phosphate content by employing ^{32}P as an indicator, the rate of renewal of the fatty acid constituents is determined by the use of deuterium[62] as indicator, and the new-formation of the choline content by applying ^{15}N as a tracer[63]. A molecule can clearly be renewed in various ways.

Site of formation of phosphorus compounds in the organism

Origin of yolk phosphatides

We shall first consider the site of formation of some constituents of the hen's egg. Where in the organism are the phosphatides found in the yolk synthesized? This question can be answered by comparing the specific activity of phosphatide P extracted from the yolk and from the different organs a few hours after administration of labelled sodium phosphate[64].

The results of an experiment in which the hen was killed 5 hours after subcutaneous injection of labelled P are seen in Table 6. The specific activities of the yolk phosphatide and the ovary phosphatide P were very low, showing that only a small part of the phosphatide molecules present in the said phosphatides had been formed within the last 5 hours. The plasma phosphatide P had a much higher specific activity than those extracted from the ovary and the yolk, while the liver phosphatide P had a higher specific activity than the plasma phosphatide P. The gradient indicating the presence of phosphatide molecules formed within the last 5 hours, thus falls off in the direction from the liver, through the plasma, into the ovary.

The conclusion that the formation of the phosphatide molecules of the plasma mainly occurs in the liver[59,64,65] is strongly supported by the results obtained in the study of fat phosphorylation in the hepatectomized dog by Chaikoff and his colleagues[66]. These authors injected labelled sodium phosphate intravenously immediately after removal of the liver. Practically no

Table 6. Specific activity of phosphatides extracted from the organs of a hen

Organ	*Relative specific activity (Activity of inorganic plasma P = 1)*
Liver	0.54
Plasma	0.43
Ovary	0.039
Yolk	0.0035
Intestine	0.11
Spleen	0.1

phosphatide ^{32}P was recovered in the plasma as late as 3–6 hours after extirpation of the liver; at these times 0.4 percent of the injected ^{32}P had been incorporated into phosphatides of both kidneys and about an equal amount into the whole small intestine.

Considerations similar to those applied to the origin of the phosphorus compounds of the yolk were used in an investigation of the phosphorus compounds of milk[67]. As seen in Table 7, the milk phosphatides were found to have a much higher specific activity than the plasma phosphatides, indicating that the phosphatides must enter the milk from a source other than the plasma and must thus have been synthesized to a large extent in the mammary gland. The determination of the specific activity of the mammary gland phosphatides revealed a very high value, even higher than those found for the kidney and liver phosphatide P.

One often encounters the view that the milk fat originates from the phosphatides of the blood, which are decomposed into fatty acid and inorganic P in the mammary gland. The inorganic P present in the milk should, according

Table 7. Specific activity of the phosphatide P extracted from the organs of a goat $4\frac{1}{2}$ hours after administration of labelled sodium phosphate

Fraction	*Specific activity*
Milk	0.09
Plasma	0.02
Corpuscles	0.01
Mammary gland	0.13
Liver	0.09
Kidney	0.11
Plasma inorganic P	1.48

to this view, originate from phosphatide P. The fat content of goat's milk amounts to about 3 percent. Taking the ratio of fatty acid to phosphorus to be 20 : 1 in plasma phosphatides, the production of 3 percent fatty acid from phosphatides would set free 0.15 percent of inorganic phosphorus. This being about the inorganic P content of the milk, almost all inorganic P of the milk should originate from plasma phosphatide. A few hours after the administration of labelled phosphate, the milk phosphatides are only slightly active, while the milk inorganic P shows a strong activity. This is a decisive argument against the above view. The high activity already found for the milk inorganic P in the early stages of the experiment, is only compatible with the assumption that the milk inorganic P is derived from the plasma inorganic P. The latter acquires a high activity soon after subcutaneous injection of labelled sodium phosphate.

As a further example we may mention the origin of the phosphorus compounds in the chick embryo[68]. Physiological sodium chloride solution (0.1 ml) containing traces of labelled sodium phosphate was injected into fertilized eggs. Several days after incubation, the phosphatides and other phosphorus compounds were isolated in turn from the embryo and the yolk, their activity and their phosphorus content being determined. As seen in Table 8,

Table 8. Specific activity of P extracted from the hen's egg incubated for 18 days

		Specific activity
Yolk residue	Acid-soluble P	1.56
	Phosphatide P	0.016
	Protein P	0.058
Embryo	Skeleton inorganic P	1.66
	Phosphatide P	1.59
	Protein P	1.44

the specific activity of the embryo phosphatide P is very different from that of the yolk phosphatide P.

While the phosphatides of the yolk are scarcely active, the phosphatides extracted from the embryo are found to have a very strong activity. The phosphatide molecules present in the embryo must obviously have been newly synthesized. Similar considerations apply to the protein phosphorus present in the embryo.

Reaction path

The path taken by organically bound phosphate radicals in glycolytic processes was investigated by using labelled compounds prepared under the action of enzymes present in muscle juice or yeast. When labelled adenyl phosphate was added to fresh muscle pulp in which glycolysis occurred, no formation of active inorganic phosphate was found to take place, but active phosphate was detected in the Harden-Young ester formed during alcoholic fermentation.

In a study of the interaction of labelled adenosine triphosphoric acid with non-labelled hexose monophosphoric acid ester, which leads to the formation of fructose 1,6-diphosphoric acid ester, the labelled phosphate given off by the adenosine triphosphoric acid was found to be exclusively present in the fructose 1,6-diphosphoric ester. The fact that the free phosphate formed according to the equation:

adenosine triphosphoric acid + hexose 6-monophosphoric ester + H_2O → fructose 1,6-diphosphoric ester + adenylic acid + phosphate

was found to be inactive, indicates that the free phosphate originated exclusively from the hexose 6-monophosphoric ester[69].

When both hydrogen and labelled phosphate were transferred, neither of the two stable radicals of the cozymase molecule was found to be replaced by active phosphate. A similar negative result was obtained for the reaction

3-phosphoglyceric acid → 2-phosphoglyceric acid

in the presence of active phosphate and also for the conversion of glucose monophosphoric acid into glucose hexaphosphoric acid in the presence of active phosphate. The ester fractions were found[70] not to have taken up ^{32}P.

Does a less pronounced formation of new molecules take place simultaneously with the autolysis observed in tissue slices? By the employment of isotopic indicators this question can be answered. On shaking liver, kidney or brain slices for a few hours with a Ringer solution containing ^{32}P at 37° C, Chaikoff and his colleagues[71] found that the phosphatides isolated from the tissue slices contained ^{32}P; hence, side by side with an autolysis of the phosphatides in the tissue slices, an appreciable formation of labelled phosphatides also takes place.

The formation of labelled nucleic acid in slices of Jensen's rat sarcoma was likewise obtained[72] in the investigation of the formation of desoxyribose nu-

cleic acid *in vitro*, when these slices were shaken with labelled blood or labelled Ringer solution. About 0.1 percent of the desoxyribose nucleic acid molecules present in the tissue slices were found to be labelled after the lapse of 4 hours; these molecules had accordingly been formed during the experiment. The presence of hydrogen sulphide, azide or carbon monoxide inhibits the formation of labelled phosphatides. Addition of cyanide, fluoride or monoiodoacetate to labelled blood or labelled Ringer solution is also found to inhibit the formation of labelled nucleic acid.

Dynamic state of body constituents

The most remarkable result obtained in the study of the application of isotopic indicators is perhaps the discovery of the dynamic state of the body constituents. The molecules building up the plant or animal organism are incessantly renewed. In the course of this renewal, not only the atoms and molecules taken up with the food participate, but atoms and molecules located in one organ or in one type of molecule will soon be found in another organ or in another type of molecule present in the same or in another organ. A phosphate radical taken up with the food may first participate in the phosphorylation of glucose in the intestinal mucosa, soon afterwards pass into the circulation as free phosphate, enter a red corpuscle, become incorporated with an adenosine triphosphoric acid molecule, participate in a glycolytic process going on in the corpuscle, return to the circulation, penetrate into the liver cells, participate in the formation of a phosphatide molecule, after a short interval enter the circulation in this form, penetrate into the spleen, and leave this organ after some time as a constituent of a lymphocyte. We may meet the phosphate radical again as a constituent of the plasma, from which it may find its way into the skeleton. Being incorporated in the uppermost molecular layer of the skeleton, it will have a good chance of being replaced by other phosphate radicals of the plasma or the lymph, but it may also have the good fortune to find a more or less lasting abode in the skeleton. This will be the case when it becomes embedded in a newly formed apatite-like bone crystallite.

There are indications that, in the growing organism, the rate of new formation of the molecules is still greater than in a fully grown organism. It was found, for example, by making use of heavy nitrogen, kindly put at our disposal by Professor Urey, as an indicator, that in « old» leaves of the sun-

flower, which did not develop further during the experiment, 12 percent of the protein molecules present were renewed within 12 days. In growing leaves, the replacement of old protein molecules was found to take place at a higher rate[73].

Schoenheimer and Rittenberg[34] have shown, by applying labelled nitrogen, that the peptide linkages in the proteins of the animal tissue are opened and reclosed with great ease. They found that the protein molecules in the living body continually change and renew their structures. This discovery is one of the most surprising and outstanding results arrived at with isotopic indicators.

Excretion studies

Chemical analyses of the food and of the excreta permit the determination of the extent to which the organism is in balance. Chemical methods, however, cannot determine to what degree the substances found in the faeces originate from undigested food and to what extent they have been carried into the digestive tract, coming from the body proper in the form of digestive juices. This problem can be solved under strictly physiological conditions with the aid of isotopic indicators.

The simplest procedure is the following[74]. At a suitable time after administration of labelled sodium phosphate, we determine the specific activity of the urine P and that of the faeces P. Both originate from the blood plasma and, provided that we wait for a sufficient time, the specific activity of the P compounds carried into the digestive tract from the body will be about equal to that of the urine P. If the faeces P were entirely of endogenous origin, it should show a specific activity equal to that of the urine P. If we find the faeces P to be less active than the urine P, the active faeces P of endogenous origin must have been diluted by non-active P. Since the sole source of non-active P is the diet, the ratio of the specific activities of the faeces P and urine P tells us to what extent the endogenous faeces P has been diluted by food P.

The ratio 100 $\times$ specific activity of faeces P/specific activity of urine P gives the percentage of P in the faeces which originates from the body proper. In the case of human subjects, $\frac{3}{4}$ to $\frac{4}{5}$ of the P present in the faeces was found to originate from non-absorbed P.

Labelled red corpuscles

As seen in Fig. 7[75], labelled phosphate penetrates at a fairly slow rate into the red corpuscles. On entering the corpuscles, however, the newly arrived phosphate ions participate rapidly in the formation of acid-soluble organic phosphorus compounds which occur in a comparatively high concentration in the corpuscles. The formation of new acid-soluble phosphorus compounds in the corpuscles is largely associated with glycolytic processes occurring there and is attended by the destruction of an equal or almost equal number of « old » molecules. As a result of these processes, the specific activity of the labile P atoms of adenosine triphosphoric acid and that of the P of some other compounds will soon acquire a specific activity almost as high as that shown by the free P of the corpuscles, but much lower than that of the free P of the plasma. This fact and the fairly slow rate of penetration of phosphate through the corpuscle wall explain the low rate of loss of ^{32}P by labelled corpuscles when brought into contact with unlabelled plasma, and make possible the application of such labelled corpuscles in the determination of the total circulating red corpuscle content of the organism[76,77]. A detailed investigation of the erythrocyte content of human subjects, making use of labelled corpuscles, was carried out by Nylin[78,88]. He estimated, furthermore, the rate at which injected blood and the circulating blood are homogeneously mixed. Fig. 8 shows the result obtained by Nylin in an experiment

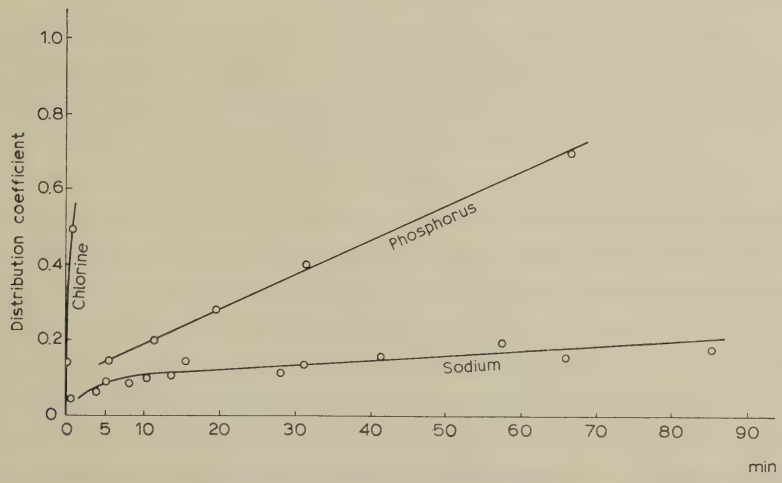

Fig. 7. Distribution of labelled ions between corpuscles and plasma of equal weight at 37°.

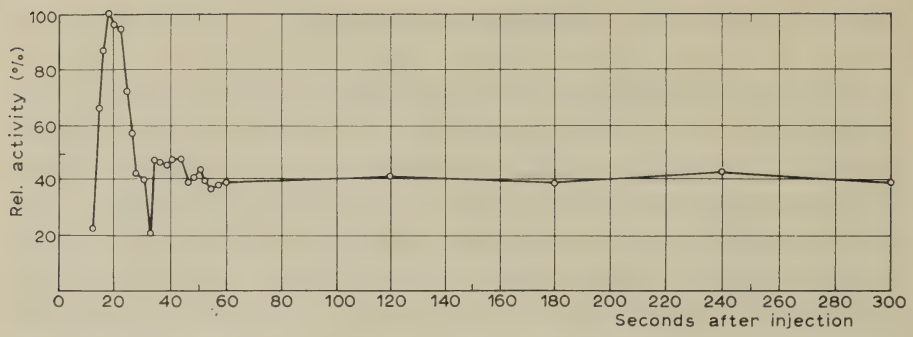

Fig. 8. Change of activity of the arterial corpuscles with time following intravenous injection of labelled corpuscles (G. Nylin).

where homogeneous distribution of the injected blood took only 60 seconds.

Corpuscles can also be labelled by introduction of radio-iron into the corpuscle haemoglobin. Such corpuscles were used by Hahn and his colleagues[79] in the determination of the red-corpuscle content of the dog. Radio-iron has a much longer half-life period than has radio-phosphorus; such « iron-labelled » corpuscles remain labelled for a much longer time than « phosphorus-labelled » corpuscles. While, however, the latter can easily be obtained by shaking blood with labelled phosphate, the former can be made only in the living organism, a fact which, together with the relatively great difficulty of obtaining radio-iron, restricts the applicability of iron-labelled corpuscles in the determination of the erythrocyte volume.

The determination of the total corpuscle volume of the organism demands only that the labelled corpuscles retain their labelling for some minutes; the determination of the life cycle of the corpuscles requires, however, the use of marked corpuscles which conserve their labelling for weeks. No worker has yet succeeded in achieving a labelling of mammalian red corpuscles that fulfils this condition. Iron-labelled corpuscles, although remaining labelled for a sufficient time, were found by Hahn and his colleagues[89] not to be suited to the purpose in hand. The life of the red corpuscles of the hen, however, was determined[80], making use of phosphorus-labelled corpuscles. In contradistinction to mammalian corpuscles, avian corpuscles contain large amounts of desoxyribose nucleic acid, and the nucleic acid molecules were found to remain unchanged throughout the life of the corpuscles. The newly formed corpuscles of a hen to which labelled phosphate is administered contain labelled desoxyribose nucleic acid.

By daily injection of labelled phosphate, the activity of the plasma phos-

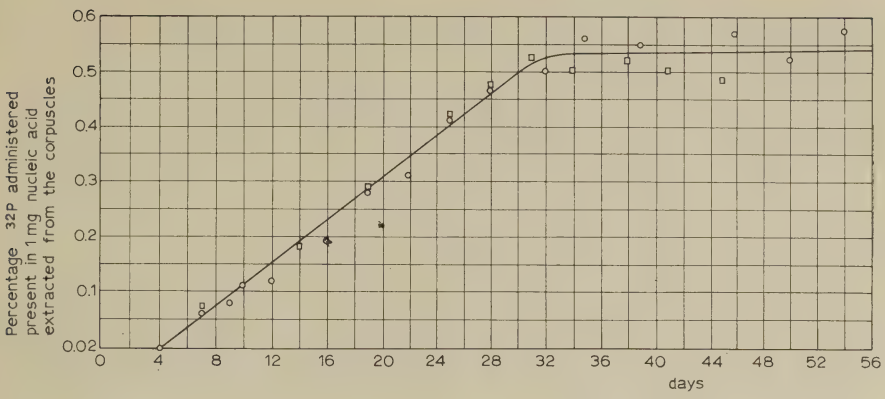

Fig. 9. Life cycle of the red corpuscles of the hen.

phate is kept at a constant level, and at suitable intervals the specific activity of the nucleic acid P extracted from the corpuscles is determined. Fig. 9 illustrates the results obtained, including the fact that, after the lapse of about 33 days, the specific activity of the nucleic acid P became constant. This indicates that all corpuscles present in the circulation of the hen were formed during the experiment. In the corpuscle samples taken in the course of the first four days, only minute amounts of labelled nucleic acid were found to be present. This may be interpreted by supposing that the formation of the corpuscles in the marrow, up to the point of their release into the circulation, requires four days; 3.5 percent of the corpuscle content of the hen is thus built up daily.

I have attempted to give a short review of the earliest applications of isotopic indicators and to discuss a few examples of their earlier and more recent employment. Their use may be much extended in the time to come.

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Biography

George de Hevesy was born in Budapest on August 1st, 1885, the son of Louis de Hevesy, Court Counsellor and Eugénie, née Baroness Schosberger. After matriculating at the Gymnasium of the Piarist Order in 1903 he studied at Budapest University and Berlin Technical University and he gained his doctor's degree at the University of Freiburg im Breisgau in 1908. He worked for two years as an assistant at the Institute of Physical Chemistry, Technical University of Switzerland before having a short spell with Professor Fritz Haber when he was able to witness much of the fundamental work of Haber and Rossignol on ammonia synthesis. He travelled to England in 1910 to study under Professor Ernest Rutherford at Manchester. He interrupted early in 1913 his studies to carry out jointly with Frederic Paneth the first radioactive-tracer experiment at the Vienna Institute of Radium Research. During his stay in Vienna he obtained the Venia Legendi in the University of Budapest. In 1915 he was drafted into the Austrian-Hungarian Army. After the end of the war he was teaching for 6 months in the University of Budapest and left the spring of 1919 for Copenhagen to discuss his future activities at Niels Bohr's Institute which was to be erected. In 1920 he settled in Copenhagen.

Six years later he returned to Freiburg as Professor of Physical Chemistry. In 1930 he was appointed Baker Lecturer at Cornell University, Ithaca. Four years later he took up again his activities at Niels Bohr's Institute which he terminated in 1952. He was domiciled in Stockholm since 1943 and was an Associate of the Institute of Research in Organic Chemistry. In 1949 he was elected Franqui Professor in the University of Ghent. In his retirement, he remains an active scientific associate of the University of Stockholm.

His early investigations involved a study of the chemical behaviour of molten salts and his introduction to practical radiochemistry came in Rutherford's laboratories at Manchester. His work there, and later in Vienna and Budapest, mainly concerned the investigation and use of radium and lead isotopes.

In Copenhagen, de Hevesy's researches were initially concerned with iso-

topic separations and in 1923, together with Coster, he discovered the element hafnium. He was responsible for pioneer work in the use of isotopic indicators both in inorganic and life sciences and later, in Freiburg, he was involved in the first clinical use of isotopes. On his return to Copenhagen, he demonstrated the formation of new artificially radioactive isotopes and subsequently introduced a method of activation analysis based on neutron bombardment of the element to be investigated. This method was to replace X-ray analysis with fluorescent X-rays which he introduced during his stay in Freiburg. The year 1934 saw the beginning of numerous investigations in the field of plant and animal physiology, using labelled atoms: these researches were supported by generous grants made by the Carlsberg Foundation, the Rask-Ørsted Foundation, the Rockefeller Foundation and others. His work in Sweden has continued on the same lines and he has studied, amongst other things, the effect of X-rays on the formation of nucleic acid in tumours and in normal organs, and iron transport in healthy and cancerous organisms; this work is supported by the Swedish State Research Council and the Wallenberg Foundation.

Professor de Hevesy is the author of several important books on radiochemistry and his many scientific papers are valuable and accurate records of devoted work. He was awarded the Cannizaro Prize (Academy of Sciences, Rome) in 1929, he was the Copley Medallist (Royal Society, London) in 1949, Faraday Medallist in 1950, Baily Medallist in 1951 and Silvanus Thompson Medallist in 1955. In 1959 he received the Ford Foundation's Atoms for Peace Award Medal, in 1961 the Niels Bohr Medal and the Rosenberger Medal of the University of Chicago. Honorary degrees conferred upon Professor de Hevesy include Doctor of Philosophy, Uppsala, Freiburg, and Copenhagen; Doctor of Science, Ghent, Liège, London, and Capetown; and Doctor of Medicine, São Paulo, Rio de Janeiro, Turin, and Freiburg. He is a Fellow of the Royal Society (London), the Swedish Academy of Sciences, Gothenburg Academy, and eleven other scientific academies. He is Honorary Fellow of the Chemical Society (London), the Royal Institution (London), the British Institute of Radiology, the Finnish Chemical Society, the German Bunsen Society, the German Physiological Society, the Chemical Society of Japan, and the American Society of Nuclear Medicine. In addition, he holds honorary memberships of many more learned societies.

Professor de Hevesy married Pia Riis in 1924. They have one son and three daughters.

Chemistry 1944

OTTO HAHN

«*for his discovery of the fission of heavy nuclei*»

Chemistry 1944

Presentation Speech by Professor A. Westgren, Chairman of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesty, Your Royal Highnesses, Ladies and Gentlemen.

When chemical compounds form or decompose, interaction takes place between the outer parts of the electron shells. In view of the fact that, up to recent times, chemistry was nearly entirely concerned with the study of the combining of atoms and their release from combined units, it can be said that up till recently it was the science of the peripheral parts of the atom. But today a new discipline has grown up, that of nuclear chemistry, which deals with the centre parts of atoms, their nuclei, and which to judge from its recent achievements, promises to revolutionize science.

An atomic nucleus is a very small thing. Rutherford found that its diameter is about ten thousand times smaller than that of the atom, or about one billionth (10^{-12}) of a centimetre. And yet, by means of the particles charged with energy of the radioactive elements, particles which act as projectiles, he succeeded in detaching small fragments from certain nuclei. The fragments detached in this way were found to be hydrogen nuclei or protons, which showed that these atomic nuclei, despite their minute dimensions, were composite structures made up of protons. Later, Joliot and his wife Irène Joliot-Curie studied in greater detail what happens when different kinds of elements are exposed to radiation by positive particles rich in energy. Here transmutations of elements may occur, but the atoms which then form are generally unstable and break up spontaneously with the emission of different kinds of elementary particles.

Fermi used the neutron discovered by Chadwick as a projectile to obtain nuclear syntheses. The neutron has the same mass as the proton, but as its name indicates it differs from it through bearing no charge. Thus it is not repelled by the positive atomic nuclei and will combine with them more easily than the projectiles with a positive charge used previously. In this way Fermi was able to produce a large number of new kinds of radioactive atoms.

All these researches into nuclear chemistry were concerned with relatively slight modifications to the mass of the reactive nuclei. Here it was simply a question of the addition or loss of different sorts of elementary particles. The

reaction processes discovered by Otto Hahn are of quite a different nature. They involve the splitting of heavy atomic nuclei into two parts of more or less equal size.

In collaboration with Lise Meitner, with whom he has worked for nearly thirty years, Hahn studied from 1936 to 1938 the products obtained by projecting neutrons on to the heaviest elements, thorium and uranium. According to Fermi, elements would appear which would form a continuation of the Periodic Table of the elements. Hahn and Meitner believed they could confirm this assumption. But towards the end of 1938, Hahn, in an investigation carried out with one of his young colleagues, F. Strassmann, found that one of the products formed through the reaction of uranium with neutrons and which had been assumed to be a kind of radium, behaved chemically in fact like barium. In January 1939 Hahn announced this discovery and expressed in very discreet terms the daring opinion that on being allied with neutrons, the atoms of the heaviest elements could split in half as it were and produce elements belonging to the middle of the Periodic Table of the elements. After a month he was able to provide proof of his theory, which was confirmed almost simultaneously in research carried out in different parts of the world by scientists using different methods.

Hahn's discovery caused great surprise and evoked lively interest among the world's scientists. It was immediately made the object of important theoretical investigations by Lise Meitner and Frisch, who based their study on the theory of the structure of atomic nuclei developed by Bohr. These investigators pointed out that nuclear fission should take place with an enormous generation of energy, due to the conversion of matter into energy. Calculations showed that the fragments produced in this break-up would disperse in all directions with immense force. Frisch demonstrated this experimentally. In connection with Joliot's observation that certain products of nuclear fission break up with the emission of neutrons, this discovery indicated that it was possible by splitting uranium to produce a chain reaction generating a very large amount of energy. The outlook for later research thus became very promising.

Without equal in the art of the chemical identification of radioactive elements in minute quantities, Hahn, together with his colleagues, paved the way for the chemical research which had to be carried out on the numerous products of the splitting of heavy atomic nuclei. Fission can be carried out in many ways, depending on the structure of the reactive nuclei and the energy of the splitting neutrons. The primary products of fission are unstable

and they gradually decompose, emitting elementary particles, so that each of them acts as the starting-off point for more or less long series of different sorts of atoms. So far, the presence has been demonstrated of about one hundred bodies which are the direct or indirect products of the processes of the splitting of matter; these products are connected with 25 elements which lie between selenium and praseodymium in the Periodic Table of the elements.

The discovery of nuclear fission is very momentous and indeed dangerous, but even more, it is full of promise. In autumn 1943, Hahn read a paper to the Swedish Academy of Sciences on his latest work in nuclear chemistry, and there referred to the possibility of splitting uranium by means of a chain reaction. In this process such enormous quantities of energy would be produced in a short instant that the effect would exceed any explosion phenomenon so far known. Hahn doubted however whether it was possible to surmount the technical difficulties involved. «Providence has not wanted the trees to reach to the sky», he said, and his hearers guessed from the passion in his voice that he wished that this conquest of atomic energy had been made at a much later date. He certainly shuddered at the thought that the atomic bomb was nearer at hand than the use of atomic energy for peaceful purposes.

Hahn's work has been inspired throughout by an invincible desire to solve the problems which he has encountered. Unlike Prometheus, who gave fire to Man, he has never dreamed of giving Man control over atomic energy. May humanity weigh deeply the responsibility which the gift of this discovery has imposed on it. Then this will be a blessing and a step towards the improvement of the conditions of human life.

The Academy of Sciences has decided to award to Professor Hahn the Nobel Prize in Chemistry for the year 1944 in reward for his discovery of the fission of heavy atomic nuclei. Professor Hahn has expressed his gratitude, but he has informed us that he is regrettably unable to attend this ceremony. Therefore the decision of the Academy cannot at present be implemented by the awarding of the prize.

Professor A. Tiselius addressed the following words to Professor Otto Hahn, on December 13, 1946

Professor Hahn. At the Nobel ceremony held on 10th December, 1945, you were unfortunately unable to be here in person to receive your Nobel

Prize. On that occasion, however, a detailed account of the results of your researches was given by the President of the Nobel Committee for Chemistry. I must therefore restrict myself today to the expression of our great pleasure at the fact that you are able to attend today in person to receive your prize and our congratulations. The discovery of the fission of heavy nuclei has led to consequences of such a nature that all of us, indeed the whole of humanity, look forward with great expectations, but at the same time with great dread, to further developments. I am convinced, Professor, that just as your great discovery has been a result of your far-reaching researches on atomic nuclei, irrespective of any eventual practical applications, the further ardent development of research in this field as a consequence of your work will be of particularly great pleasure to you. With regard to its practical application I am also sure that you share all our hopes that this application will serve in the end as a blessing to mankind.

Professor Otto Hahn. Whilst offering you the sincere congratulations of the Academy, I ask you to receive from the hands of His Majesty the King the Nobel Prize for Chemistry for the year 1944.

Otto Hahn

From the natural transmutations of uranium to its artificial fission

Nobel Lecture, December 13, 1946

The year 1946 marked a jubilee in the history of the chemical element, uranium. Fifty years earlier, in the spring of 1896, Henri Becquerel had discovered the remarkable radiation phenomena of this element, which were at that time grouped together under the name of radioactivity.

For more than 100 years, uranium, discovered by W. H. Klaproth in 1789, had had a quiet existence as a somewhat rare but not particularly interesting element. After its inclusion in the Periodic System by D. Mendeleev and Lothar Meyer, it was distinguished from all the other elements in one particular respect: it occupied the highest place in the table of the elements. As yet, however, that did not have any particular significance.

We know today that it is just this position of uranium at the highest place of the then known chemical elements which gives it the important properties by which it is distinguished from all other elements.

The echo of Becquerel's fundamental observations on the radioactivity of uranium in scientific circles was at first fairly weak. Two years later, however, they acquired an exceptional importance when the Curies succeeded in separating from uranium minerals two active substances, polonium and radium, of which the latter appeared to be several million times stronger than the same weight of uranium.

It was only a few years before the first surprising property of this «radiating» substance was explained. The radioactive elements decompose according to definite rules into other active elements with different chemical and physical properties, simultaneously emitting corpuscular particles, the α- and β-rays. The α-rays were characterized by Rutherford as positively charged helium nuclei, after it had been shown earlier that the β-rays consisted of negative elementary particles, the so-called electrons.

With the hypothesis, suggested by Rutherford and Soddy in 1902, of the disintegration of the atoms, the postulate of the indivisibility of chemical elements had to be abandoned.

Parallel with the investigation of the radioactive disintegration processes

of uranium, which produce radium, radium emanation, the so-called active deposits, and polonium, research was carried out on thorium, at that time the second highest element of the Periodic Table. From thorium were obtained, in an exactly similar manner, strongly active disintegration products: radiothorium, mesothorium, thorium X, etc. There was also a third series of disintegration products, the actinium series, which however also started from uranium.

By the systematic study of the penetration of α-particles through thin layers of material, Rutherford in 1911 was able to propose his model of the nuclei of chemical atoms. According to this view, the atoms of the elements consist of the positively charged nucleus, representing practically the whole mass of the atom, around which move, at a relatively great distance, electrons which neutralize the nuclear charge. The charge of the nucleus undoubtedly represents the position of the element in the Periodic System.

The radioactive disintegration reactions are thus nuclear changes which are not affected by the usual processes of physics or chemistry.

We are so certain of the unchangeability of these natural laws of radioactive breakdown, that from the speed of the changes we can construct a sort of «geological clock». Actually the final substance which is produced from uranium by way of these many active intermediate products is stable lead; from the quantity of the latter formed in the originally pure uranium mineral, it is possible to calculate the age of the mineral and therefore also of the geological deposit in which it has crystallized out. The same holds for thorium, which also produces as final product a kind of lead, although different from the «uranium lead».

Investigation proceeded further. Again the α-particles of radioactive substances formed the means for the solution of problems of nuclear physics. Following Rutherford's nuclear model, these particles were recognized as free helium nuclei. With their relatively large mass of 4 (compared with the small electron mass of about 1/1800) and their initial velocity of up to 15,000 km/sec, they formed the bullets with which it might be possible to blast a way to the nuclei of the atoms, inaccessible to other means. And again it was the genius Rutherford who made the next discovery, which was to have extremely important results. In 1919 he was able to show that by bombardment of nitrogen with the energetic α-particles of radium C, an α-particle, that is a helium nucleus, was captured by the nitrogen nucleus, and a hydrogen nucleus – a proton – left the newly formed nucleus.

The process is represented by the equation:

$$^{14}_{7}\mathrm{N} + {}^{4}_{2}\mathrm{He} \rightarrow {}^{17}_{8}\mathrm{O} + {}^{1}_{1}\mathrm{H} + \text{energy}$$

In this equation the lower indices represent the nuclear charges, the upper ones the nuclear masses (atomic weights). The remaining energy is given up in the form of kinetic energy to the released proton, which shows that the latter is derived from the nucleus.

Here for the first time was an artificial transmutation of atoms, actually a building-up of atoms, since an oxygen of mass 17 was produced from a nitrogen of mass 14.

In the following years a large number of reactions following the same pattern were discovered. On account of the positive charge of the α-particle this type of reaction did not succeed in bringing about nuclear transmutations of the heavy elements, since their high nuclear charge of positive sign repelled the α-particle sufficiently to prevent its penetration into the nucleus.

The year 1932 brought further important discoveries: the positron, heavy hydrogen, and – last but not least – the neutron. The discovery of the neutron was the result of detective work carried out in three countries. The initial impulse was given by Bothe and Becker in Germany, who were able to detect an exceptionally penetrating radiation, believed by them to consist of γ-rays, on exposing beryllium to α-particles. Then the Joliot-Curies in France proved that the «γ-rays» which appeared in the experiments of Bothe and Becker set free hydrogen nuclei (protons) with great energy from hydrogen-containing compounds, which is impossible for γ-rays. Finally Chadwick in England gave the final explanation of these results: in addition to γ-rays there occurred uncharged neutral atomic nuclei with a mass of 1, which he called neutrons. The course of the reaction is:

$$^{9}_{4}\mathrm{Be} + {}^{4}_{2}\mathrm{He} \rightarrow {}^{12}_{6}\mathrm{C} + {}^{1}_{0}\mathrm{n} + \gamma$$

On the discovery of neutrons, the phenomenon of isotopy, known for a number of years, could readily be explained. The chemical elements are composed of protons and neutrons. The number of charged protons determines the chemical nature of the element: the sum of protons and neutrons determines the atomic weight. Thus a larger or smaller number of neutrons does not affect the chemical nature of the element, but merely produces isotopic kinds of atoms such as had been known for a long time in the different isotopes of lead, many mercury isotopes, two of chlorine, etc. Heavy hydrogen, discovered in 1932, is similarly an isotope of ordinary hydrogen. It has in its nucleus both a proton and a neutron, and thus has a mass of 2

instead of 1. The highest chemical element known at that time, uranium, is also not a simple element, but contains isotopes of which ^{238}U is the parent of the radium series and ^{235}U that of the protactinium and actinium series.

Soon was it seen that neutrons would form projectiles specially suited for the transmutation of atoms: they have no charge and are thus not repelled by the positive nuclei of the elements. While however the (α, p) decompositions of Rutherford – (α, p) is the abbreviated formula for a reaction in which an α-particle is taken up and a proton split off – nearly always produce stable kinds of atoms which occur naturally, in the (α, n) changes – the taking up of an α-particle and emission of a neutron – the Joliot-Curies had observed an entirely new phenomenon. In 1934 they found that, on irradiation of certain elements with α-particles, not only neutrons but also positrons, that is positive electrons, appeared, even after the irradiation with α-particles had ceased. The emission of positrons fell off in the same way as had already been observed in the decay of natural radioactive elements. Artificial active elements had been produced. The first elements with which this «artificial» activity on irradiation had been observed were boron and aluminium.

From boron was produced an active nitrogen; from aluminium an active phosphorus; and in their turn these were transformed into carbon and silicon respectively, with emission of positrons, according to the reactions

$$^{10}_{5}B + {}^{4}_{2}He \rightarrow {}^{13}_{7}N^{*} + {}^{1}_{0}n; \quad {}^{13}_{7}N^{*} \xrightarrow[10\,min]{\beta+} {}^{13}_{6}C$$

$$^{27}_{13}Al + {}^{4}_{2}He \rightarrow {}^{30}_{15}P^{*} + {}^{1}_{0}n; \quad {}^{30}_{15}P^{*} \xrightarrow[2.2\,min]{\beta+} {}^{30}_{14}Si$$

(* indicates a radioactive isotope)

A new and large field for further investigation was hereby opened. At the same time the experimental possibilities for carrying out nuclear physical investigations were fundamentally expanded.

While so far the particles from natural radioactive elements had been the sole means of producing artificial nuclear reactions, high-voltage installations – van de Graaff generators and the cyclotron – could now be used in addition. More and more intensive irradiations could be carried out, and more and more new reactions could be realized.

For the time being, however, radium–beryllium mixtures continued to be used, since they were very convenient, though perhaps not very intensive, sources of neutrons for further investigations, especially of the newly discovered artificial radioactivity. Such sources of neutrons were prepared by thor-

ough mixing of a well-dried, finely-powdered radium salt with the finest possible beryllium powder, and sealing the mixture into airtight glass or metal tubes. Uranium itself was only of importance because radium was obtained from it.

It was especially the Italian scientist Fermi who first realized the great importance of neutrons for the production of nuclear reactions: Fermi and his co-workers irradiated practically all of the elements of the Periodic System with neutrons, and made numerous artificial radioactive elements.

Generally, the course of the reaction was such that, especially when working with slow neutrons*, the neutrons were captured by the nucleus. Thereby in many cases an unstable isotope of the irradiated element was produced, passing into the next higher element with emission of β-rays. This latter was generally a stable atom occurring among the usual chemical elements, e.g.

$$^{127}_{53}\mathrm{I} + {}^{1}_{0}\mathrm{n} \rightarrow {}^{128}_{53}\mathrm{I}^{*} + \gamma; \quad {}^{128}_{53}\mathrm{I}^{*} \xrightarrow[26\,\mathrm{min}]{\beta} {}^{128}_{54}\mathrm{Xe}$$

Fermi and his co-workers continued their tests through the whole of the Periodic System up to uranium. Here also they discovered many transmutations produced by neutrons, including some very rapid ones. They proceeded from the obvious assumption that initially there are produced artificial, active, short-living uranium isotopes; as these emit β-rays Fermi inferred the production of so-called «transuraniums», representatives of the element 93 which is not known naturally, and possibly even of the still higher element 94.

Fermi's proofs were not accepted everywhere. It was pointed out that for example in the case of the so-called 13-minute element – that detected with the greatest certainty – the possibility of its being an isotope of element 91, i.e. protactinium, could not be ruled out**.

At this point Lise Meitner and I decided to repeat Fermi's experiments in order to decide whether the 13-minute element was a protactinium isotope or not. This decision was taken the more readily since, by the discovery of protactinium (1917), we were familiar with its chemical properties. More-

* As Fermi first showed, such slow neutrons, of low energy, can be obtained from the primary high-energy neutrons by passing them through substances with a high content of hydrogen. The kinetic energy of the neutrons is then transferred to the hydrogen nuclei in elastic collisions.

** From another direction (Ida Noddack) the objection was raised that all the elements of the Periodic System must first be excluded before it was possible to draw the conclusion that an element 93 had been obtained. This objection was not taken seriously as it appeared to be in opposition to all physical views of nuclear physics.

over, a β-radiating isotope of element 91 was well known to us in the form of uranium Z, discovered by myself, which had the favourable half-life of 6.7 hours, and was available from uranium salts.

With the help of the « indicator method » we were able to prove without doubt that the 13-minute element of Fermi was neither a protactinium isotope, nor a uranium, actinium, or thorium. In accordance with the position of science at the time, Fermi's assertion should be correct, and the 13-minute element a representative of the element 93, that is a « transuranium ».

We should point out here that other possibilities did not occur to anyone at that time. Since the discovery of the neutron and the application of artificial sources of radiation, a large number of most unusual nuclear reactions had been discovered; the products were always either isotopes of the irradiated substances, or their next, or at most next-but-one, neighbours in the Periodic System; the possibility of a breakdown of heavy atomic nuclei into various light ones was considered as completely excluded.

With the tests on Fermi's 13-minute element and the checking of other, rather less certain, results of Fermi, we found (later in co-operation with F. Strassmann) that the phenomena associated with the irradiation of the highest element of the Periodic System were much more complicated than had originally been supposed. Fermi and his co-workers had already, in their first communication, described two short-life β-radiating kinds of atoms (half-life 10 sec and 40 sec), which they naturally considered to be artificial isotopes of uranium produced from the original uranium by the capture of neutrons. Lise Meitner and I found, in addition, a substance with a half-life of 23 minutes, which we conclusively identified as an artificial radioactive uranium isotope. With Fermi's substances of short life, the isotopy with uranium can only be assumed, but not proved. The 23-minute element occurred without any other radiation conditions in a so-called « resonance process ».

As the result of many years of work, we (Hahn, Meitner, and Strassmann) had finally obtained a great number of artificial active kinds of atoms, which all appeared to be formed directly or indirectly by β-radiation from the supposed short-living uranium isotopes, and which therefore must all represent so-called transuraniums – elements higher than uranium.

According to their chemical behaviour, these could be classified into various groups, and, since in many cases the gradual production from β-radiating parent substances could be directly observed, decay schemes were drawn up extending to elements 95 and 96. In so far as the work was repeated by others, the results were always confirmed.

Independently of the transuranium investigations of Hahn, Meitner, and Strassmann just mentioned, Curie and Savitch described in 1937 and 1938 a so-called 3.5-hour substance which they had obtained by irradiation of uranium with neutrons, and of which the chemical properties could not readily be determined. According to Curie and Savitch, the substance appeared to be a rare earth, but was not actinium; it had more resemblance to lanthanum, and could only be separated from the latter by « fractional crystallization ». With some hesitation Curie and Savitch decided to include the substance in the transuranium series, but the possibilities put forward by them appeared difficult to understand and unsatisfactory.

As this 3.5-hour element had been included with the transuraniums, I, together with Strassmann, tried to obtain it. After careful experiments we arrived at remarkable results, which may be formulated approximately as follows: « In addition to the transuraniums described by Hahn, Meitner, and Strassmann, there are produced by two successive α-emissions three artificial, β-active radium isotopes with different half-life times, which in their turn change into artificial β-active actinium isotopes ». The conclusion that radium isotopes had been produced was the only one possible since, according to the chemical properties, only barium and radium could be considered. Barium was, according to the physical viewpoint of the time, impossible, and thus only radium was left.

The separation of this active group was performed by means of a barium precipitate; not however in the form of barium sulphate, which with its large surface strongly adsorbs other elements, but, on the suggestion of Strassmann, as barium chloride, which crystallizes very well from concentrated hydrochloric acid and which precipitates uncontaminated by other substances.

At the same time the production of radium under these conditions of radiation was very remarkable: α-decompositions had never been observed with neutrons low in energy, and yet here, as with the transuraniums, a number of isotopes appeared simultaneously.

The experiments were continued in various directions. The preparations were, however, always very weak and the α-rays of the most stable of the new isotopes were so strongly absorbed that thicker layers could only be investigated with poor yields of radiation. An attempt was therefore made to separate the artificial « radium » as far as possible from the barium added as carrier, in order to obtain coatings permitting easier measurement. This was done by fractional crystallization using the method of Madame Curie,

a method with which we had been thoroughly familiar over a number of years. About 30 years previously I, together with Lise Meitner, had separated the radium isotope mesothorium from barium by fractional crystallization. More recently, with the assistance of a number of co-workers, the laws governing the formation of mixed crystals between radium and barium salts had been systematically investigated.

The attempts to separate our artificial «radium isotopes» from barium in this way were unsuccessful; no enrichment of the «radium» was obtained. It was natural to ascribe this lack of success to the exceptionally low intensity of our preparations. It was always a question of merely a few thousands of atoms, which could only be detected as individual particles by the Geiger-Müller counter. Such a small number of atoms could be carried away by the great excess of inactive barium without any increase or decrease being perceptible, even if the barium was precipitated in the form of barium chloride, which precipitates in a very pure form.

In order to check this, we repeated the same tests with a weak intensity of the natural radium isotopes mesothorium and thorium X. These substances were freed from every trace of their parent substance and decay products with the greatest care and, by systematic dilution, preparations were made which were only just detectable with the Geiger-Müller counter. Crystallizations were carried out with the chlorides, bromides and chromates, always with the corresponding barium salt as carrier.

The result was, as was to be expected for radium, that mesothorium and thorium X were concentrated in the first fractions of the salts named, and in fact in quantities such as we should expect from our previous experience. This proved that the few atoms of natural radium isotopes also behaved in exactly the same manner as strong preparations.

Finally we proceeded to direct «indicator tests». We mixed the pure natural radium isotopes with our artificial «radium» isotopes, also previously freed from their decay products, and fractionated the mixture in the same way as before. The result was that the natural radium isotopes could be separated from barium, but the artificial ones could not.

We checked the results in still another way. If the artificial alkaline earth isotopes were radium, then the decay products produced directly through β-emission should consist of actinium: from the element 88 should be produced the element 89. If on the other hand it was barium, then lanthanum should be formed: from element 56 the next higher element 57. With the aid of the pure actinium isotope mesothorium-2 we carried out an «indicator

test» by mixing mesothorium-2 with one of the known primary decay products of artificial radium isotopes, and then carrying out the chemical separation of actinium and lanthanum by the method of Madame Curie. During the fractionation of lanthanum oxalate with actinium, the latter accumulates in the final fractions. This actually occurred with the actinium isotope mesothorium-2. The decay product of our so-called «radium isotope» however remained with the lanthanum. The artificial rare earth, which had been considered to be actinium, was really lanthanum. Thus it was established that the alkaline earth isotope, which we had believed to be radium, was in fact an artificial active barium; the lanthanum could have been produced only from barium and not from radium.

In order to make quite certain, we carried out a so-called «cycle» with barium. The most stable of the active isotopes, now identified as barium, was freed from active decay products and other impurities by recrystallization with inactive barium; one quarter of the total quantity was kept for comparison, and three quarters were subjected to the following cycle of barium precipitations: Ba-chloride → Ba-succinate → Ba-nitrate → Ba-carbonate → Ba-ferrimannite → Ba-chloride. After passing through this series of compounds, many of which crystallized beautifully, the resulting barium chloride and the recrystallized comparison preparation were measured alternately using the same counter, with equal weights and equal thicknesses of layers. The initial activity and the increase as the result of further formation of the active lanthanum were the same for both preparations, within the limits of error: the crystallization of so many and such different salts had produced no separation of the active barium from the carrier. It could only be concluded that the active product and the carrier were chemically identical, that is, barium.

In the first communication on these tests, which «were in opposition to all the phenomena observed up to the present in nuclear physics» (January 6th, 1939), the indicator tests mentioned had not been entirely completed, and we had therefore expressed ourselves cautiously. As a second partner in the new process we assumed an element with an atomic weight of about 100, as in that case the combined atomic weights would be that of uranium, «for example 138 + 101 (e.g. element 43) gives 239!»

After the completion of the measurements in hand, and of the «cycle», the possibility of error was still further excluded.

This completion of the tests and the above-mentioned «cycle» appeared in a second communication (February 10th, 1939). This also described the

splitting of the element thorium and its confirmation with the aid of indicator tests analogous to those described above. Here also reference was made to the detection of an inert gas and an alkali metal derived from it; the nature of the gas was recognized, and its separation from uranium accomplished by means of a current of air passed over the uranium during the irradiation. An active strontium and an active yttrium were identified in the uranium itself.

Immediately after the first publication on the production of barium from uranium, there appeared as a first communication an article by Lise Meitner and O. R. Frisch in which the possibility of a breakdown of heavy atomic nuclei into two lighter ones, with total charges equal to that of the original nucleus, was explained with the aid of Bohr's model of the original nucleus. Meitner and Frisch also estimated the exceptionally high energy output to be expected from this reaction, from the curve of the mass deficiencies of the elements in the Periodic Table. The great repulsive energy of the fragments produced by the splitting was first demonstrated experimentally by Frisch and shortly afterwards by F. Joliot. Meitner and Frisch soon proved that the active breakdown products, previously considered to be transuraniums, were in fact not transuraniums but fragments produced by splitting. They were able to accumulate these by «repulsion» outside the radiated uranium.

In quick succession there appeared a whole series of publications from European and American nuclear physics institutes, confirming and expanding the tests described.

Thus the process proceeds in such a way that the nucleus of the uranium with a charge of 92 is split into two nuclei of moderate size*. If one of these is barium, which has a nuclear charge of 56, there must be produced at the same time a krypton with a nuclear charge of 36. Together these nuclei add up to 92. Both have however, as may easily be seen from the masses of uranium and of the stable isotopes of barium and krypton which occur naturally, too great a mass, and thus an excess of neutrons. They should therefore pass over into stable elements with higher nuclear charges, with emission of β-rays; and in fact, as our later experiments showed, sometimes achieve stability by way of a great number of unstable intermediate decay products.

The highest stable krypton isotope has a mass of 86. In uranium fission there is produced, among other atoms, an unstable krypton with mass 88.

* The expression «nuclear fission» (*Kernspaltung; fission nucléaire*) is due to Meitner and Frisch.

Uranium 235 is responsible for the fission induced by thermal neutrons, as Bohr was the first to see; this fission forms by far the larger part. If there are no side reactions then the mass of the other fission product belonging to the krypton 88, that is of the barium, should be 236 − 88 = 148. As the highest stable barium isotope has a mass of 138, the first-mentioned product is not less than 10 units heavier. Strassmann and myself had already noted, in our second communication, the possibility that neutrons were set free in the fission process. That this was in fact the case was first established experimentally by F. Joliot.

The investigations continued at a rapid pace, both from the physical and the chemical side. Only a year after the first communication on the production of barium from uranium, there appeared in the *Reviews of Modern Physics* (U.S.A.) a bibliography on the splitting of heavy nuclei (*Nuclear Fission*, by L. A. Turner) in which nearly one hundred publications in this sphere were mentioned.

During the Second World War, the very confusing fission reactions were systematically investigated in the Kaiser Wilhelm Institute for Chemistry with a view to their chemical disentanglement, and numerous new reactions were discovered. Japanese investigators found that, when fast neutrons were used, the fission of uranium proceeded more symmetrically than with slow

Element	*Natural isotopes*	*Isotopes occurring in the uranium fission*
54 Xenon	124, 126, 128, 129, 130, 131, 132, 133, 134, 136	139 (unstable) together with
38 Strontium	84, 85, 87, 88	97 (unstable)
92		236
36 Krypton	78, 80, 82, 83, 84, 86	88 (unstable)
56 Barium	130, 132, 134, 135, 136, 137, 138	together with 148 (unstable)
92		236

92 = nuclear charge of uranium = sum of the nuclear charges of the fragments
236 = mass number of uranium 235 plus 1 neutron

Table 1. Atomic weights of some natural isotopes, and of artificial isotopes produced by nuclear fission, if the excess of neutrons is not released.

ones. At the beginning of 1945 we were able to make a table (Table 3) in which were collected, as direct or indirect products of uranium fission, 25 different elements, ranging from 35 (bromine) to 59 (praseodymium), in the form of about 100 active kinds of atoms. The active atoms, believed by us up to 1939 to be transuraniums, were all fission products and their active successors, and not elements with atomic number higher than uranium!

	Br	Kr	Rb	Sr	Y	Zr	Nb	Mo
	35	36	37	38	39	40	41	42
83	140^{min} / 113^{min} stab.							
84	30^{min} → stab.							
85	3^{min} → 4.6^{h}	stab.						
86	∘	stab.						
87	50^{sec} → 75^{min} → $6 \cdot 10^{10a}$ →	stab.						
88		175^{min} → 18^{min} →	stab.					
89		2.5^{min} → 15^{min} →	55^{d} →	stab.				
90		? →	? →	5^{a} →	60^{h} →	stab.		
91		? →	? →	10^{h} →	50^{min} / 57^{d} →	stab.		
92		? →	80^{sec} →	2.7^{h} →	3.5^{h} →	stab.		
93						65^{d} →	55^{d} stab.	
94		? →	? →	2^{min} →	20^{min} →	stab.		
95						17^{h} →	75^{min}	
96		? →	? →	7^{min} →	11.6^{h} →	stab.		
97		? →	? →	~ 8^{h} →	> 100^{d} →	26^{d} →	∘ →	stab.

Table 2. Fission products of uranium between elements 35 and 42.

From the nature of the problem, the physical work proceeded in a different direction. Especially important in this connection was the above-mentioned investigation of Joliot in which he proved experimentally, in the spring of 1939, that in the fission process, neutrons appeared in addition to the (always two) new elements.

Since by the action of neutrons on uranium, fresh neutrons are liberated, the latter, if they meet uranium atoms, produce further fissions, in their turn.

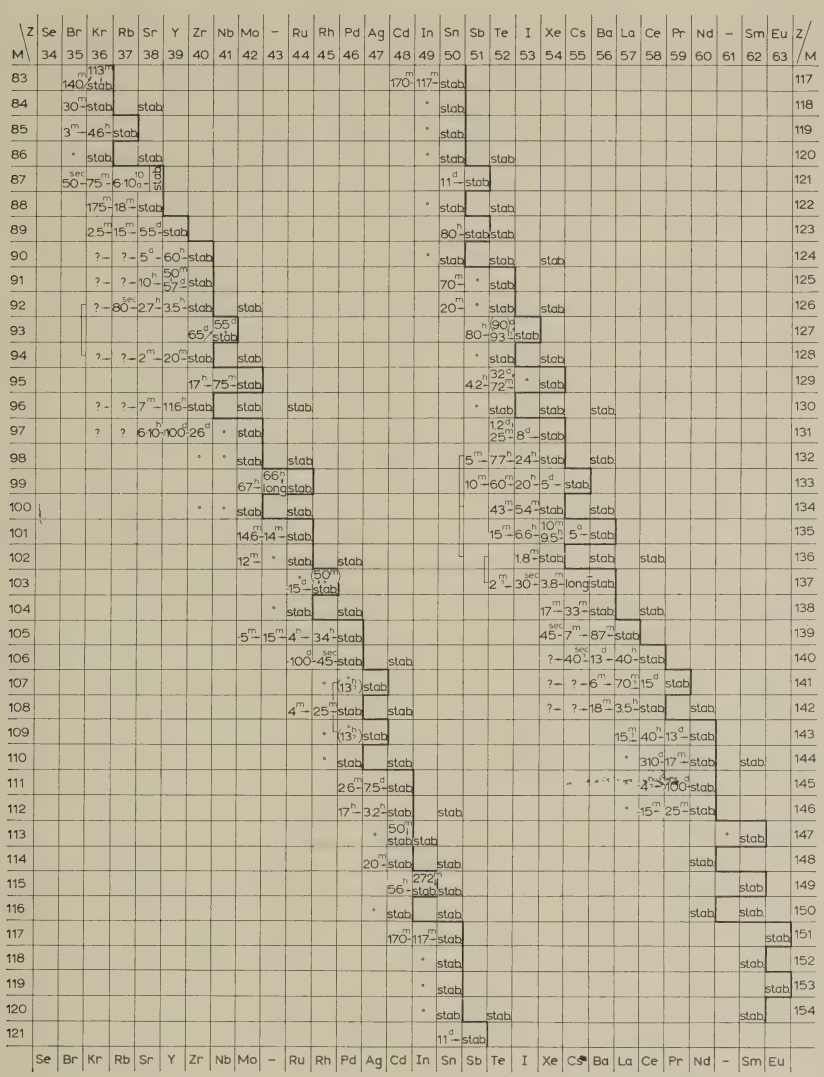


Table 3. Summary of the fission products of uranium known at the Kaiser Wilhelm Institute for Chemistry by early 1945.
(s = seconds; m = minutes; h = hours; d = days; a = years)

If more than one fresh neutron is produced, and the process is so arranged that all the fresh neutrons strike uranium atoms, then we have a chain of continuously renewing fission reactions which, like an avalanche started by a snowball, can attain enormous dimensions. Thereby the practical application of atomic energy first came into the range of possibility. S. Flügge, then attached to the Kaiser Wilhelm Institute for Chemistry, was the first to refer to this.

About 10 years ago, Joliot concluded his Nobel Lecture with the following words: « If, turning to the past, we cast a glance at the progress achieved by Science at an ever-increasing pace, we are entitled to think that scientists, building up or shattering elements at will, will be able to bring about transmutations of an explosive type, true chemical chain reactions. If such transmutations do succeed in spreading in matter, the enormous liberation of usable energy can be imagined. But, unfortunately, if the contagion spreads to all the elements of our planet, the consequences of unloosing such a cataclysm can only be viewed with apprehension. Astronomers sometimes observe that a star of medium magnitude increases suddenly in size; a star invisible to the naked eye may become very brilliant and visible without any telescope – the appearance of a Nova. This sudden flaring up of the star is perhaps due to transmutations of an explosive character like those which our wandering imagination is perceiving now – a process that the investigators will no doubt attempt to realize while taking, we hope, the necessary precautions! »

What was ten years ago only a figment of our « wandering imagination », has already become to some extent a threatening reality. The energy of nuclear physical reactions has been given into men's hands. Shall it be used for the assistance of free scientific thought, for social improvement and the betterment of the living conditions of mankind? Or will it be misused to destroy what mankind has built up in thousands of years? The answer must be given without hesitation, and undoubtedly the scientists of the world will strive towards the first alternative.

Postscript

In the preceding paragraphs we have given a general outline of how the investigation of the natural radioactivity of uranium led to the artificial splitting of that element. But this does not exhaust the possibilities of ura-

nium. Uranium consists mainly of two isotopes with atomic weights 235 and 238. The first is present in the mixture only to the extent of $\frac{1}{140}$ part; nevertheless the fission process described above, which is caused with special violence by «slow» neutrons, is to be ascribed chiefly to its rare isotope. It has already been mentioned above that Lise Meitner and the present writer were able to identify, after the irradiation of uranium with neutrons, a substance having a half-life of 23 minutes as undoubtedly a uranium isotope. (The short-lived isotopes, thought first by Fermi and later by ourselves to be artificial uranium isotopes, were actually fission products.) The 23-minute uranium was produced in a so-called resonance process by neutrons of a certain velocity. Since this substance emits β-rays, a representative of element 93, that is an actual transuranium, *must* be produced. Although we sought for it, we were unable to detect it in the very weak preparations which we had at the time. Later it was identified in the United States as a β-emitter with a half-life of 2.3 days. Thus it has an atomic weight of 239. Since this true transuranium 93 emits β-rays in turn, it must be concluded that an element 94, a further transuranium, is formed. This is a relatively long-living α-emitting substance (half-life 24,000 years). The American scientist Seaborg gave it the name plutonium, calling element 93 neptunium. A second isotope of element 93 also occurs, it is produced in another way from uranium 238, namely by a so-called (n, 2n) process with the aid of neutrons of great energy: one neutron, so to speak, in passing through the uranium nucleus knocks out another one. The result is a β-emitting uranium

I. Natural

$$^{238}\mathrm{U} \xrightarrow{\alpha} \mathrm{UX_I} \xrightarrow{\beta} \ldots\ldots \mathrm{Ra} \ldots\ldots \mathrm{Pb}$$

$$^{235}\mathrm{U} \xrightarrow{\alpha} \mathrm{UY} \xrightarrow{\beta} \mathrm{Pa} \xrightarrow{\alpha} \mathrm{Ac} \ldots\ldots \mathrm{Pb}$$

II. Artificial

(i) *Fission processes* with slow and fast neutrons, up to the present 25 elements in about 100 kinds of atoms.

(ii) *Capture of a neutron*:

$$^{238}_{92}\mathrm{U} + \mathrm{n} \rightarrow {}^{239}_{92}\mathrm{U} \xrightarrow[23\,\mathrm{m}]{\beta} {}^{239}_{93}\mathrm{Np} \xrightarrow[2.3\,\mathrm{d}]{\beta} {}^{239}_{94}\mathrm{Pu} \xrightarrow[24{,}000\,\mathrm{a}]{\alpha} {}^{235}_{92}\mathrm{U} \rightarrow$$

(iii) *Loss of a neutron*:

$$^{238}_{92}\mathrm{U} + \mathrm{n} - 2\mathrm{n} \rightarrow {}^{237}_{92}\mathrm{U} \xrightarrow[6.8\,\mathrm{d}]{\beta} {}^{237}_{93}\mathrm{Np} \xrightarrow[2.25 \cdot 10^6\,\mathrm{a}]{\alpha} {}^{233}_{91}\mathrm{Pa} \rightarrow$$

Table 4. Uranium transmutations.

isotope of mass 237, which thus transforms into a second neptunium isotope of which the half-life is some millions of years.

Thus the behaviour of uranium on irradiation with neutrons of different velocities, both fast and slow, is very complicated (see Table 4). In addition to the natural splitting process, which continues during the irradiation at a speed independent of all the other reactions, the following occur:

(1) Nuclear fission with formation of numerous artificial atoms of all elements between 30 and 64.

(2) Emission of surplus neutrons during this fission process, making a chain reaction possible.

(3) The resonance capture of a neutron with a definite energy by uranium 238, with formation of uranium 239, which in its turn is transformed into the elements neptunium and plutonium.

(4) The giving up of a surplus neutron by the ^{238}U with formation of a ^{237}U, which also forms a neptunium isotope.

As, in process (1), slow (thermal) neutrons mainly attack the rare isotope ^{235}U; process (3) is a kind of competitor of process (1), since in the resonance process (3) the neutrons are captured before they have reached thermal velocities. It is a matter of experimental technique to prevent this capture as much as possible, in order to facilitate the chain reaction. On the other hand, the extra neutrons produced in the fission of process (2) give rise to the resonance process, and thereby to the formation of plutonium. If this latter element is obtained in sufficient quantity by means of a slow controlled chain reaction carried out on a large scale (in a so-called « pile »), and is separated from uranium, then it can also act as carrier of a chain reaction. The same holds for pure ^{235}U after it has been separated from the isotope 238, since with ^{235}U the resonance process, which interrupts the chain reaction, does not occur.

Both uranium 235 and plutonium are made in the United States. The result was the bombing of Hiroshima and Nagasaki.

Biography

Otto Hahn was born on 8th March, 1879, at Frankfurt-on-Main. He attended the secondary high school there until he matriculated.

From 1897 Hahn studied chemistry at Marburg and Munich, taking his doctorate examination in 1901 at Marburg and submitting to Professor Theodor Zincke a thesis on organic chemistry.

He obtained a post as assistant in the Chemical Institute at Marburg, staying there two years, after which he worked under Sir William Ramsay at University College, London, from the autumn of 1904 to the following summer. His work here was rewarded by the discovery of a new radioactive substance, radiothorium, while working on the preparation of pure radium salts.

From the autumn of 1905 to the summer of the following year Hahn was at the Physical Institute of McGill University, Montreal (Canada) working under Professor Ernest Rutherford. Here he discovered radioactinium and conducted investigations with Rutherford on alpha-rays of radiothorium and radioactinium.

On his return to Europe Hahn moved to Berlin, to the Chemical Institute (Emil Fischer) of the University and there he qualified as a university lecturer in the spring of 1907, which year also saw his discovery of mesothorium.

At the end of 1907, Dr. Lise Meitner came to Berlin from Vienna and then began more than thirty years' collaboration. Their joint work embraced: investigations on beta-rays, their absorbability, magnetic spectra, etc.; use of the radioactive recoil, discovered shortly before by Hahn, to obtain new radioactive transformation products.

Between 1914 and 1918 Hahn's work was interrupted by his service in the First World War, but he resumed his research with Professor Meitner in 1918, and discovered protactinium, the long-lived mother substance of the actinium series. Hahn's own particular sphere was chemistry and he further discovered uranium Z, the first case of a nuclear isomerism of radioactive kinds of atoms. Using radioactive methods he investigated the absorp-

tion and precipitation of the smallest quantities of substances, normal and abnormal formation of crystals, etc. Hahn used the emanation method to test substances superficially rich or poor, and he elaborated the strontium method to determine the age of geological periods.

Following the discovery of artificial radioactivity by M. and Mme. Joliot-Curie and the use of neutrons by Fermi for atomic nuclear processes, Hahn again collaborated with Professor Meitner and afterwards with Dr. Strassmann on the processes of irradiating uranium and thorium with neutrons.

Hahn and Prof. Meitner had also worked together on the discovery of an artificially active uranium isotope, which represents the basic substance of the elements neptunium and plutonium, first revealed later in America.

Hahn's work has won recognition in many learned circles. In 1912 he became scientific member of the Kaiser Wilhelm Institute for Chemistry and has been Director of this Institute since 1928. 1933 saw his appointment as Visiting Professor at Cornell University, Ithaca, New York. From 1st April, 1946, he has officiated as President of the Kaiser Wilhelm Society and from 28th February, 1948, has served as President of the Max Planck Society in Western Germany, being created Honorary President of the same Society in May, 1960.

His most spectacular discovery came at the end of 1938. While working jointly with Dr. Strassmann, Hahn discovered the fission of uranium and thorium in medium heavy atomic nuclei and his first work on these subjects appeared on 6th January and 10th February, 1939, in *Naturwissenschaften*. Since that time and until 1944 Hahn continued investigation on the proof and separation of many elements and kinds of atoms which arise through fission.

Hahn has been granted membership of the Academies of Berlin, Göttingen, Munich, Halle, Stockholm, Vienna, Boston, Madrid, Helsinki, Lisbon, Mainz, Rome (Vatican), Allahabad, Copenhagen, and the Indian Academy of Sciences.

In 1913 Hahn married Edith, *née* Junghans and they had one son, Hanno, born in 1922, killed by accident in 1960.

Chemistry 1945

ARTTURI ILMARI VIRTANEN

«for his research and inventions in agricultural and nutrition chemistry, especially for his fodder preservation method»

Chemistry 1945

Presentation Speech by Professor A. Westgren, Chairman of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesty, Royal Highnesses, Ladies and Gentlemen.

In our northern latitudes we have to feed our most useful domestic animals in winter with preserved fodder, generally hay. But for a long time it has been common knowledge that hay alone is insufficient to keep the animals in perfect condition. To be able even in winter to produce good quality milk in sufficient quantity our cows need concentrated fodder. Thus, so far they have been fed in most cases on oil cakes imported from warmer countries. Obviously, replacing these products imported from other countries by an indigenous feeding stuff would offer appreciable economic advantages for our agriculture.

This is precisely the achievement of the AIV method elaborated by Virtanen and denoted by the initials of his name. Therefore the Swedish Academy of Sciences has decided that his research and discoveries in the field of agricultural and nutrition chemistry, and particularly his method of preserving animal fodder, have qualified him for the Nobel Prize for Chemistry, 1945.

For a long time Virtanen had been seeking to make what contribution he could to improving the supply of feeding stuffs in his country. With the indomitable tenacity which marks the sons of Finland he never lost sight of that objective and stubbornly persisted in carrying out his research programme.

Leguminous plants such as clover, vetch and lucern are green fodder which, when harvested in due season, provide cattle with the vitamins and proteins necessary for them to achieve full production capacity. In seeking to achieve by rational and economic cultivation a green fodder with the maximum protein content Virtanen was led to study the conditions of nitrogen assimilation and of protein formation in vegetable organisms. Displaying great ingenuity, he attempted to solve the difficult problem of the process whereby the leguminous plants fix atmospheric nitrogen by means of bacteria contained in their tubers. The original aim of these studies has still not been achieved but their results are already valuable and hold promise of being very important.

In his attempts to improve the availability of feeding stuffs in Finland, Virtanen also founded on purely theoretical data his method of preserving green fodder, a method which avoids protein loss and minimizes vitamin loss.

It had long been known that the addition of organic or mineral acids to ensilage inhibited the breathing of the vegetable cells as well as all the fermentation processes. There have been frequent endeavours to turn this phenomenon to practical account. However, these attempts were carried out empirically and without serious study of the conditions for conserving the nutrient value of the fodder, or for using it as an animal feeding stuff. By his systematic, thorough studies Virtanen was the first to solve this problem.

To preserve fodder Virtanen uses hydrochloric acid with the addition of sulphuric acid. After painstaking, protracted studies he determined the limiting degrees within which the acidity must be kept in order to achieve the required result. The following beneficial results are simultaneously obtained. The breathing of the plant cells, which consume readily soluble carbohydrates is reduced to a minimum, particularly if the fodder is well stacked. The lactic-acid fermentation ceases or remains at an insignificant level. The butyric-acid fermentation, which decreases the attractiveness of the fodder and lowers the quality of the milk, ceases. Protein decomposition, which is apt to cause heavy financial losses, ceases almost altogether. The content of vitamin A and carotin is maintained and vitamin B and vitamin C are also well preserved. Vitamin-rich milk and butter are very important for public health and AIV fodder provides an effective means of improving this supply of vitamins. Because of these fodders « summer milk can be produced the whole year round ». AIV fodder has no deleterious effect on the animals which readily consume it. Some weeks after the silo has been charged the mineral acids are neutralized and fixed in the form of salts by the basic products contained in the fodder, while harmless organic acids are liberated. AIV fodder has frequently assisted in improving the condition of animals, their fertility and resistance to disease.

One merit of the AIV method is that it also enables second crops of grass gathered in the autumn to be ensilated, regardless of the atmospheric conditions, and thus permits economic use of this fodder which, more often than not, cannot be used as hay.

Virtanen is not an office-bound scientist. Himself a farmer, he has tried out the most appropriate ways of applying his ensilation system.

This system has become established in Finland. A flight nowadays over

the farming areas of southern Finland shows one or more pits near almost every farm. The observer may perhaps think that they are the traces left by the bombardments of the last war. Nothing of the sort. They are silos filled with AIV fodder. In Sweden too the AIV method is becoming increasingly appreciated. Between 1932, the year when it was applied for the first time, and today, the quantities of green fodder prepared by the AIV method have doubled or tripled every four years. Last year they rose to 295,000 tons. The use of the method has increased appreciably during recent years in Denmark and Great Britain. It is also applied in Norway and Holland. In America as well it has stimulated growing interest and is each year being increasingly practised. In America and Germany large quantities of silage are prepared by methods which may be considered as variants of the AIV method. Virtanen's studies provide a firm basis for future research in the matter of silage.

Professor Virtanen. I do not think I am wrong to say that it is in your ardent patriotism that we must look for the most powerful force inspiring your great scientific achievement. During your years of effort Finland remained for you as for the noble commandant in Runeberg's poem: «the sullen, needy, humble, and holy Fatherland». By your work you have confirmed that the person who places his sincerity and a tireless zeal at the service of his kin and of his country, without thought of himself and without seeking for personal profit, also serves the interests of humanity. Perhaps your eyes are fixed more attentively than ever on the difficult biochemical problems which you have set yourself to solve and to which you have made so many important contributions. For that reason we are certain that your studies will again bear fresh fruit. It is a joy for our Academy to be able to give you new means of attaining the aims which you have set yourself. We warmly wish you prosperity and I would ask you to receive from the hands of His Majesty the King, the Nobel Prize for Chemistry for the year 1945.

Artturi I. Virtanen

The biological fixation of nitrogen and the preservation of fodder in agriculture, and their importance to human nutrition

Nobel Lecture, December 12, 1945

Nitrogen is an essential constituent of proteins, the central building bricks of all living matter. Appropriate nitrogen nutrition is thus essential to both plants and animals. The molecular nitrogen of the atmosphere is the main supply of nitrogen for our earth and from it originates all the nitrogen found in living nature. Since all the higher and the majority of the lower plants, and the entire animal kingdom are unable to assimilate atmospheric nitrogen, it is a precondition for life that the nitrogen must be fixed in some way by other substances to form compounds which can be assimilated by plants. Nitrogen nutrition is frequently a minimum requirement for growth in the natural state. In effective agriculture, where we must of course try to achieve the maximum plant production per unit of area, one of the most important tasks is to provide plants with a sufficient supply of nitrogen.

In nature, molecular nitrogen is fixed to a certain degree by electrical discharges in the atmosphere. The main nitrogen fixation, however, is produced by the action of certain micro-organisms. Some of these fix nitrogen where they live freely in the ground and have suitable carbon compounds available, while others fix nitrogen only in symbiosis with a host plant, from which they obtain their carbon nutrition. Of the latter, the bacteria of leguminous plants are the most important. They are also predominantly the most effective nitrogen suppliers in agriculture, if we do but know how to make use of them.

Today it is also possible to fix atmospheric nitrogen industrially by ammonia synthesis based mainly on Haber's research. The idea that agricultural nitrogen requirements should largely be covered by this process is quite general even amongst chemists, but this is not the case. If we consider the agriculture of the entire world, the proportion of synthetic nitrogenous fertilizers in relation to the nitrogen contained in the crops is very low (possibly 0.3%). The following statistical calculation carried out in 1936 in con-

nection with the agricultural nitrogen balance in the U.S.A. gives us a clear picture of the situation.

According to this, the quantity of industrially manufactured nitrogenous fertilizers used annually in the U.S.A. was equal to 0.48 million tons of nitrogen or just a few percent of the total quantity of nitrogen supplied to the soil, 16.45 million tons. The quantity of nitrogen fixed by micro-organisms, on the other hand, was 9.87 million tons, of which 5.6 million tons was due to leguminous plant bacteria. It is also clear from these statistics that the nitrogen content of arable land in the U.S.A. drops annually, i.e., the plants

Table 1. Nitrogen balance of arable acreage in the U.S.A. according to calculations carried out by Lipman and Conybeare in 1936.

Quantity of nitrogen annually supplied to arable acreage (millions of tons)		*Quantity of nitrogen annually taken from arable acreage (millions of tons)*	
In stable manure	2.57	In crops	4.61
In nitrogenous fertilizers	0.48	By erosion	5.00
By rain and artificial irrigation	3.57	By washing out	5.00
By biological nitrogen fixation (Symbiotic N-fixation 5.46; non-symbiotic N-fixation 4.37)	9.83	By grazing and stock breeding	9.05
Total	16.45	Total	23.66

are using up the soil's nitrogen reserve. The annual nitrogen loss in arable land in the U.S.A. according to the above calculation was 7.21 million tons.

With regard to *protein production*, the European countries, and particularly those carrying on intensive milk production, have to rely on imports. Although these countries have high crop yields, mainly due to intensive application of fertilizers, including nitrogen fertilizers, the ratio of protein to carbohydrate in the crops is not such that the fodder would be suitable for relatively high milk production without extra protein. The protein content is sufficiently high only in the case of the legumes, and in fact the protein content may even be much greater than necessary, so that the legumes occupy a special position in protein production. Protein production is thus closely related to biological nitrogen fixation.

Coverage of agricultural protein requirements, however, depends not only on the production of protein-rich fodder but also on the *preservation* thereof, since in the cold and temperate zones cattle cannot obtain fresh

fodder direct from the land during the cold seasons, while in the hot zones drought is a similar obstacle. The basic problem for intensive livestock production is therefore the problem of preserving the fodder. Enormous quantities of nutritive substances, both protein and carbohydrates, are annually lost as a result of preservation losses. The fact that *the protective substances* in food, such as the vitamins and certain amino acids, are very easily destroyed, is particularly detrimental to both man and beast. Research for the development of preservation methods is therefore equally as important as increased production.

After this introduction I should now like to mention those aspects of our research which relate to agriculture and human nutrition and for which the Swedish Royal Academy of Sciences has awarded this year's Nobel Prize in Chemistry. From the very outset I should like to stress the important contribution made by my colleagues in the results we have achieved.

In the spring of 1925 in the modest laboratory of Valio, the central association of the Finnish co-operative dairies, we began research in connection with the nodule bacteria of leguminous plants. These tests clarified for me the extraordinarily vigorous growth of legumes, which live in symbiosis with the bacteria of their nodules, solely by means of atmospheric nitrogen. Although at that time I had no close experience of agriculture, the experiments that had been carried out set my imagination in motion and led me to think how it might be possible to utilize atmospheric nitrogen to promote fodder cultivation and milk production by intensive and rational legume cultivation. It was self-evident that our knowledge of the activity of legume bacteria, the factors influencing nitrogen fixation, and its chemical mechanism, must be widened as much as possible if these remarkable bacteria were to be utilized effectively. The biological nitrogen fixation was therefore the central problem at the laboratory of Valio and later at the Biochemical Research Institute, which was founded in 1931.

When I studied the literature of the subject in 1925, I found that the difficulties in preserving protein-rich legume crops were a decisive factor in limiting the possibilities of utilizing these plants agriculturally. Such fodder can generally be dried to hay only once per summer and even then the weather conditions are a decisive factor. If the fodder is prepared for ensilage, the resultant fodder is of poor quality. High preservation losses (25 to 50% of the nutritive value) are also inherent in these two methods. *Inter alia*, a large part of the valuable protein is lost. For effective utilization of legumi-

nous plants, it was therefore necessary to prepare a reliable preservation method whereby the fresh fodder could yield a high-grade end product with low losses. The fodder preservation problem was therefore incorporated in the research programme of our laboratory. If it were possible to make fuller use of symbiotic nitrogen fixation and devise effective legume cultivation while successfully solving the problem of fodder preservation, hitherto unimagined possibilities would be opened to self-supporting milk production while it would also be possible biologically to increase the nitrogen content of arable land. That was the practical object of our work. Since the problems were also integrally bound up with such an important natural phenomenon as the assimilation of gaseous nitrogen, this research was also particularly interesting from the scientific aspect.

N-fixation in the root nodules of leguminous plants

As our research progressed, it soon became apparent that it was necessary for the experimental methods to be greatly improved. Since it had been found that the leguminous plant bacteria fix nitrogen only in symbiosis with their host plants, experiments had previously been carried out by cultivating test plants inoculated with legume bacteria in open pots in a nitrogen-free or low-nitrogen medium, for example quartz sand. Under such conditions, of course, there were also other micro-organisms than legume bacteria in the medium, particularly in the region of the roots. These bacteria could, of course, also include freely living N-fixing bacteria. The test plants could therefore obtain their nitrogen nutrition in some other way than just by the co-operation of the legume bacteria. We therefore prepared a *sterile cultivation technique*, in which the plant media were free of micro-organisms unless they had been inoculated with some particular bacteria, for example a strain of legume bacteria. In the summer of 1929 we carried out the first experiments using the sterile cultivation system and it was possible to prove positively that legumes with nodules could well utilize atmospheric nitrogen without the presence of other micro-organisms, and that the nodule bacteria in symbiosis with the leguminous plants therefore fix nitrogen as Hellriegel and Willfart had assumed in 1887 following upon their classic experiments in open pots. In the summer of 1930, by the use of the sterile cultivation technique in experiments with inoculated vetch we were able to prove the presence of nitrogen compounds diffused out of the root system. We also

found that this nitrogen was of an organic nature. After we had simplified and rendered the sterile cultivation technique effective (Fig. 1), it was possible to study this secretion of nitrogen compounds more closely. We found that inoculated legumes can in certain cases secrete into the surrounding sand even greater quantities of nitrogen compounds than the host plant itself has assimilated. Special tests pointed to the proposition that the secretion was from the root nodules.

Since the secretion was greatest in young plants in which the root nodules are still in growth, there was reason to assume that the nitrogen compounds collected in the medium were *intermediate products* in protein synthesis and not decomposition products of the proteins. It was therefore important to isolate and identify these nitrogen compounds.

Examination showed that over 90% of the nitrogen diffused out was amino-nitrogen. At an early stage of growth before the flowering of peas, the majority of this amino-N originates from *l*-aspartic acid. Some of the nitrogen belongs to β-alanine, which is formed by aspartic acid by decarboxylation. The reaction is caused by the leguminous plant bacteria. Since β-alanine is a secondary splitting product of aspartic acid, it was obvious that aspartic acid is the primary secretion product. Determination of the secretion phenomenon also provided an explanation of the disputed question concerning the way in which the nitrogen fixed in the root nodules is assimilated by the host plant and what N-compound or compounds make up this «fixed nitrogen», which serves as N-nutrition for the host plant. There was immediately reason to assume that the N-fixation took place on the surfaces of the bacterial cells in the root nodules and that the resultant amino acid, probably aspartic acid, is secreted in the plant sap and is thus assimilated by the host plant. Since the quantity of root nodules and, in particular, the quantity of active contents of the nodules did not increase substantially during the period of intensive nitrogen fixation to the same extent as initially when the nodules were formed, it was clear that the bacterial cells in this phase only slightly assimilate the fixed nitrogen to build up their cell protein and that the majority is therefore utilized by the host plant. The symbiotic N-fixation is thus very economical from the aspect of the host plant, since the bacteria use for their cell material only a comparatively small quantity of the carbon compounds of the host plant.

Bond arrived at the result that approximately 90% of the nitrogen fixed in the root nodules of the soya bean is assimilated by the host plant during the best growth period. Wilson and Umbreit have found similar values. In

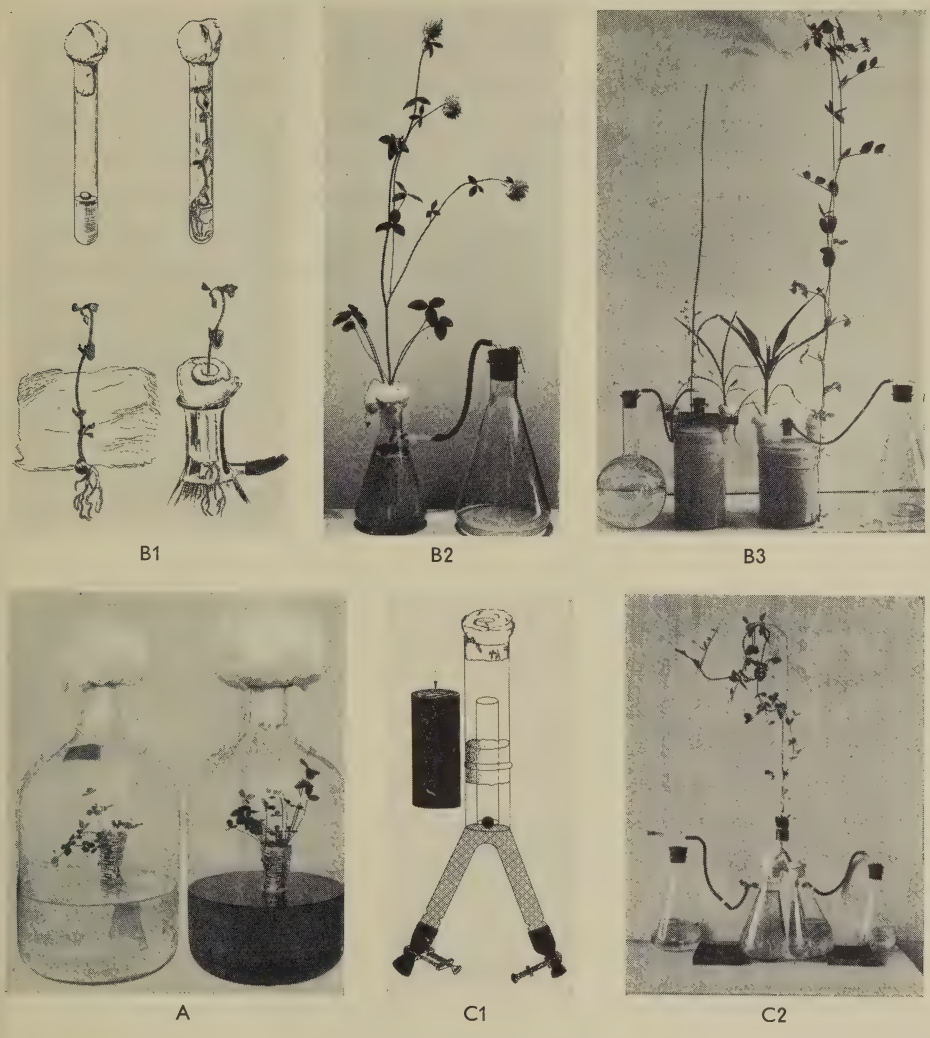


Fig. 1. Sterile cultivation system.

(*A*) An experimental arrangement from 1929. (The experimental plants grow entirely inside a 9-litre flask.)

(*B*) An experimental arrangement from 1931. (The root system of the experimental plant is in a sterilized bed, the green parts of the plant grow freely in air.)

B1: performance of the experiment; *B2*: red clover in a sterile system; *B3*: pea and maize growing in the same Woulfe flask without N-nutrition; non-inoculated pea on the left, inoculated on the right.

(*C*) «Breeches-tube» system, in which the experimental plant root system grows down in two flasks.

C1: experimental arrangement; *C2*: pea in breeches tube.

our tests with peas, the host plant proportion of the fixed nitrogen rose to 93% (Fig. 2).

With regard to the chemical mechanism of N-fixation, I initially assumed that aspartic acid was formed from fumaric acid and ammonia by the action of aspartase ($HOOC—CH=CH—COOH + NH_3 \rightleftarrows HOOC—CH_2—CH(NH_2)—COOH$), but it soon proved that aspartase did not occur in the root nodules, so that this hypothesis had to be abandoned. Since small quantities of nitrite-nitrogen, the quantity of which increased during evapora-

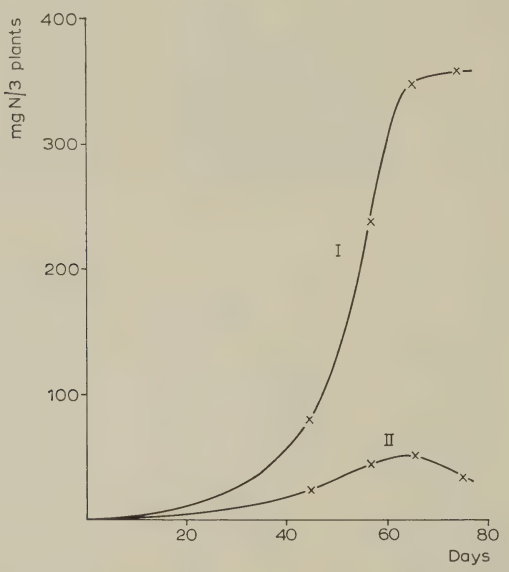


Fig. 2. Experiment with inoculated pea in quartz sand. Three plants in each pot. Three pots in each test series.
Curve I: N in host plants; *Curve II*: N in root nodules.

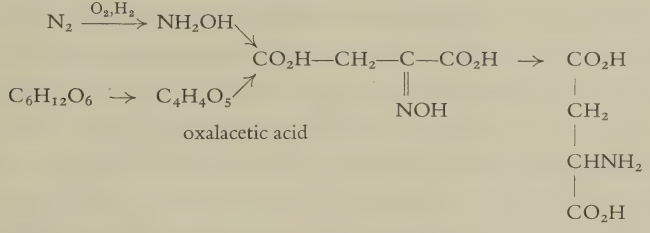
Days of growth	*Period of growth (days)*	*N-increase (mg)*		*N-increase (%)*	
		In the host plants	*In the root nodules*	*In the host plants*	*In the root nodules*
0–45	45	+ 80.6	+21.7	78.8	21.2
45–57	12	+158.5	+21.7	88.0	12.0
57–66	9	+107.8	+ 7.7	93.3	6.7
66–75	9	+ 11.8	—16.9	—	—
	Total	358.7			

tion, were found in the water extract obtained from the plant bed, my attention was drawn to another possibility.

At about the same time (1935) Endres had found oxime-N in *Azotobacter* cultures and we too were subsequently able to find it amongst the secretion products of the root nodules. The above-mentioned nitrite-N apparently occurs from decomposition of the oxime. The quantity of oxime-N was very small, about 1–2% of the total nitrogen in the extract.

It was therefore difficult to isolate and identify it. We finally obtained aspartic acid by reduction of the oxime fraction. As a result, we could conclude that oxalacetic acid oxime was included in the oxime fraction.

On the basis of these results I propounded the following hypothesis concerning the mechanism of N-fixation in the root nodules of legumes.



According to this hypothesis, the host plant would supply the carbon component, namely oxalacetic acid, with which the hydroxylamine formed from the molecular nitrogen reacts to form oxime which gives aspartic acid on reduction. It should also be mentioned that Blom had propounded a very interesting hypothesis at the beginning of the 1930's, according to which hydroxylamine would occur as an intermediate product in biological N-fixation.

The formation of aspartic acid as the primary amino acid in N-fixation is particularly interesting because according to earlier experiments (Braunstein and Kritzman, 1937) the amino group is transferred in animal tissues both from this amino acid and from its homologue, glutamic acid, to other α-keto acids by the enzyme transaminase. According to our experiments the transamination reaction with green plant material and root nodules takes place only in the system α-aminodicarboxylic acid (or ketodicarboxylic acid) + aliphatic α-ketomonocarboxylic acid (or α-aminomonocarboxylic acid) $\rightleftarrows$ α-ketodicarboxylic acid (or aminodicarboxylic acid) + α-aminomonocarboxylic acid (or ketomonocarboxylic acid). The reaction could not be brought about with α-amino or α-keto acids having a ring structure as the other com-

ponent, so that it is not possible to explain the synthesis of all amino acids by the transamination reaction.

According to our findings, growing pea plants contain about 50 γ and red clover contains about 100 γ oxalacetic acid per 1 g of fresh plant material if the analysis is carried out on a sunny day. The oxalacetic acid content drops rapidly in the dark. If pea or clover plants are left in the dark for 48 hours no oxalacetic acid can be found in them.

It is naturally not impossible that the N-fixation would only partly result in the formation of oxime nitrogen and that the reaction progresses mainly by way of ammonia and imino acid. The quantity of oxime found is very small indeed. If hydroxylamine is formed as an intermediate product in the ammonia synthesis ($N_2 \rightarrow NH_2OH \rightarrow NH_3$), the formation of oxine nitrogen in small quantities would be explained. However, there is still no direct proof that NH_3 appears as an intermediate product although one would expect a formation of ammonium nitrogen along with the hydroxylamine. If ammonia is formed in nitrogen fixation, glutamic acid would necessarily occur, since glutamic acid dehydrogenase, which was examined closely by Euler and Adler and which catalyzes the glutamic acid synthesis from ketoglutaric acid and ammonia, occurs both in the animal and plant kingdoms. We again demonstrated in our laboratory the occurrence of α-ketoglutaric acid in legumes in approximately the same quantities as oxalacetic acid. The absence of glutamic acid in the secretion products from the root nodules does not point to glutamic acid as the primary amino acid with aspartic acid, but the greater activity of glutamic acid in the transamination reaction may perhaps explain this.

Wilson and his colleagues in Madison (Wisc.), who some years previously were still very sceptical concerning the occurrence of aminodicarboxylic acids as primary amino acids in biological N-fixation, now (1945) consider from experiments performed with ^{15}N isotope that these amino acids are primary with the emphasis on glutamic acid.

We have no appreciable knowledge of the enzymes that catalyze the first stages of N-fixation ($N_2 \rightarrow NH_2OH$ or $N_2 \rightarrow NH_3$). Noteworthy experiments have, however, recently been carried out in connection with the red pigment found in root nodules. These experiments have shown that the pigment is closely bound up with N-fixation. According to Mothes and Pietz, the pigment is a derivative of dioxyphenyl alanine, while according to Kubo of Japan it is haemoglobin, which can store and transport oxygen. Kubo did not relate the pigment to N-fixation.

Owing to the war we were not able to examine the matter in our laboratory until the autumn of 1944. We first of all confirmed the accuracy of Kubo's observation concerning the nature of the pigment and proved that those root nodules that fix nitrogen always contain haemoglobin (a report to the meeting of the Finnish Academy of Sciences on January 12, 1945), which we now began to call leghaemoglobin to distinguish it from blood haemoglobin (Keilin and Wang in England also confirmed the haemoglobin nature of the red pigment in 1945). If the pigment is absent from the root nodules or if it is denatured, or if it has been changed to a green pigment, of which more later, no nitrogen fixation takes place.

Together with leghaemoglobin, brown legmethaemoglobin with trivalent iron also occurs in the root nodules. Contrary to conditions in the case of blood, the valence exchange $Fe^{II} \rightleftarrows Fe^{III}$ readily takes place in the root nodules. The oxidase enzymes in the root nodules apparently play an important part in oxidation of the iron to form leghaemoglobin. Oxalacetic acid, on the other hand, appears to be an important factor in maintaining equilibrium on the ferro-side.

When peas, soya and other legumes grow in strong light, the ferro-form is predominant in the root nodules, but when the lighting is reduced the balance gradually shifts towards the ferri-side.

Since the iron in leghaemoglobin is so easily oxidized and reduced, I began to speculate on the possibility that the N-fixation would be related to this valence exchange although the oxygen transport might possibly already explain the importance of the leghaemoglobin in nitrogen fixation. The observations by Wilson and his colleagues concerning the inhibiting effect of carbon monoxide on nitrogen fixation even in small concentrations can very easily be explained as being dependent upon the formation of a compound of the leghaemoglobin Fe^{II} iron and CO. In the first phase of N-fixation we probably have a complicated catalyst system which is not restricted to the leghaemoglobin system. However, in this context there is no reason to discuss in greater detail these questions which are still unsolved.

The haemoglobin system appears to be split in the root nodules in two different ways:

1. By denaturation, when the pigment group is separated from the protein part. The pigment is not obtained in aqueous solution from root nodules in which this change has taken place.
2. By conversion in approximately the same way as the formation of the bile pigments in the animal organism (Barkas, Lemberg). The porphyrin

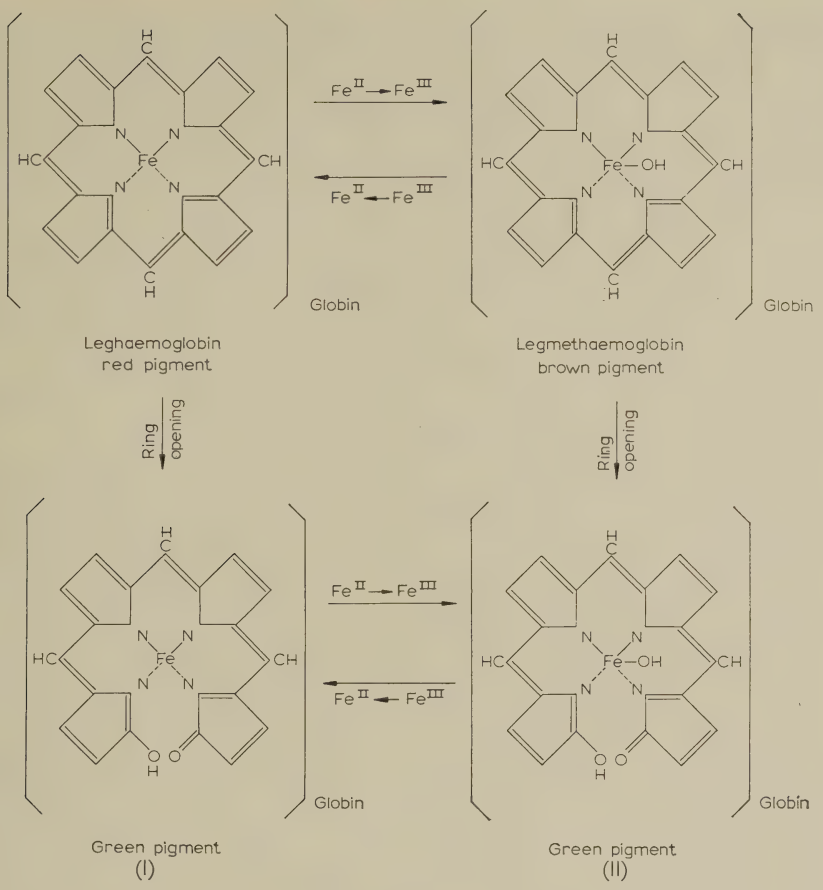
ring in these conditions is split between two pyrrol rings, whereby the combined CH-group is split off.

When the peas or soya beans ripen, the N-fixation ceases and the root nodules assume a green colour. The same change also takes place when vigorously growing legumes are kept in the dark for some days. The green pigment still contains iron although in a form easily split off with acids, and the pigment continues to be a chromoprotein although it can be easily denaturized. The iron content is the same as in leghaemoglobin. The properties of the green pigment are similar to choleglobin which was prepared by Lemberg from blood haemoglobin. Since the leghaemoglobin in the root nodules appears initially to pass over to methaemoglobin and it is only then that the green pigment appears, and since the green pigment occurs at a pH of 6.5 and less at room temperature from methaemoglobin (not from haemoglobin), if some ascorbic acid and hydrogen peroxide are supplied to the solution, it is possible that the green pigment is also formed in this way in the root nodules. The ascorbic acid content of the root nodules is sufficient to bring about this reaction. If we combine Lemberg's research with our own observations, we can provisionally give the mutual relationship shown on p. 85 between the red, brown, and green pigment in the root nodules.

The leghaemoglobin molecular weight determined by Dr. Pedersen in Svedberg's laboratory from a leghaemoglobin preparation which I sent him, and the degree of purity of which on the basis of its iron and hemin content was probably in the region of 85%, was approximately 34,000, i.e. half of the molecular weight in blood haemoglobin if there were no dissociation of the protein.

Apart from the chemical side of the nitrogen fixation problem, the more biological side also formed the subject of our continual research. Of this I will mention here only some of the problems of significance to practical agriculture.

The formation of leghaemoglobin in the root nodules is a result of symbiosis between the nodule bacteria and the host plant. The inability of the ineffective bacterial strains to fix atmospheric nitrogen is clearly due to the fact that root nodules formed by the same do not contain leghaemoglobin. Since the same bacterial strain in symbiosis with *one* host plant can form active root nodules containing leghaemoglobin, while with *another* host plant they form leghaemoglobin-free inactive root nodules (for example a bacterial strain isolated from the root nodules of *Trifolium alexandrinum* is effective in symbiosis with this species of *Trifolium*, but ineffective with



Trifolium pratense), the formation of the pigment is decisively dependent on the host plant as well. The leghaemoglobin is clearly not found in the bacterial cells themselves, because it dissolves as soon as the root nodules are crushed in a mortar. Whether the bacteria form the hemin component of the pigment and the host plant its protein component or *vice versa*, or whether the symbiotic formation of the pigment is explained in some other way is still obscure.

As a result of our sterile cultivation technique we were able positively to prove that the efficacy of individual bacterial strains is a highly constant property and that it does not change, for example, by successive passage through a host plant, as stated in the literature of the subject. The fact that several researchers arrived at this proposition is obviously due to the fact that other bacterial strains than the one in question have also been involved,

and this easily happens in experiments carried out in open vessels. This question is very important to agriculture, since the use of bacterial inoculation in the cultivation of the customary legumes on old cultivated land is based on the assumption that the bacterial strains used are very effective. If the efficacy of the bacterial strains were to vary to the extent indicated in literature, we could not refer to effective and ineffective strains. We have been able to prove positively the occurrence of quite ineffective legume bacterial strains, and that once they are allowed to penetrate into the root system they prevent the function of the effective strains. This demonstrates the importance of a correct choice of the bacterial strains used for inoculation.

Of the other results of practical importance in connection with leguminous bacteria and legumes I would give the following details:

The pH curve of the activity of legume bacteria has an optimum at pH 6.5–7.0, and this is also the optimum for the legume growth. On the acid side the activity of the legume bacteria ceases completely below pH 4.5 whereas the legume plants still grow to some extent at a pH of 4 if nitrate fertilizers are applied. In practice, however, the pH requirements of the bacteria determine the legume growth. If good leguminous plant crops are to be obtained in acid soils, the application of lime is therefore necessary.

The looseness of the earth, i.e., the air content, is of great importance to the activity of the root nodules. During rainy summers the lack of oxygen in stiff clay soils prevents the growth of peas, because the activity of the root nodules is paralyzed. By mixing a few percent of pumice stone in a stiff clay which was watered generously during the experiment, we have been able to increase the pea crop up to as much as 100% in pot tests. Also, 0.3–1% of chopped straw has in some cases resulted in a considerable crop increase. The conditions in the field are complicated because during dry weather such an addition may be detrimental as a result of excessive drying of the soil.

Determination of the importance of leghaemoglobin to the nitrogen fixation process makes it possible to obtain an idea of the activity of the root nodules in legume cultivation by examining the colours of the sections of the nodules after cutting. If the section is red, it shows that a vigorous N-fixation takes place, a brown colour indicates at least temporary reduction of the nitrogen fixation, while green sections show that the nitrogen fixation has ceased. It is not always easy to explain why chromoproteins vary from case to case, but an investigation of the question is important to the control of leguminous plant cultivation.

Research over the past 20 years has thrown a certain light on symbiotic

N-fixation. The improvements to the research methods and fundamental observations made hitherto will clearly accelerate the development of this field of research and thereby increase the possibilities of agriculture making even more effective use of atmospheric nitrogen than is now the case. Industry, which with its capital and technical resources can generally rapidly utilize scientific results and even promote research, is unfortunately not interested in biological nitrogen fixation because the latter does of course lead development «back to nature». This circumstance naturally acts as a brake on research to a certain extent.

Preservation of protein-rich crops

In the autumn of the same year (1925) as we carried out our first experiments with legume bacteria, I began to devise a reliable and effective method of preserving fresh fodder crops. The preparation of silage has undoubtedly been applied for thousands of years, as is apparent from certain Egyptian relics of antiquity, but in 1925 there was still no reliable theoretical basis for preparing silage despite comprehensive experiments, particularly during this century. The work in this field had largely been unfruitful because the tests were not based on theoretically grounded working hypotheses which could lead to generally valid results. Development was also greatly obstructed by an absence of clear principles for judging the quality of silage. The literature of the subject therefore frequently did not enable us to conclude what kind of results the method used really gives. Under such conditions it was self-evident that there would be a large number of processes which to some extent differed and that they all had their own advocates. In this connection it must be stated that G. Wiegner in Zurich in the 1920's carried out critical research work to clarify the losses of nutritive substance in various methods of preservation, and *inter alia* he found that the losses with the very much publicized electro-silo process were as high as 40–50%.

The following considerations formed the basis of our research into the preservation of fresh fodder.

The nutritive substances are broken down by the following processes in the preservation of fresh fodder:

1. By respiration of the plant cells, which first of all results in a loss of carbohydrates. This naturally takes place only at the beginning of the preservation when the plant cell respiration is still in progress.

2. By harmful fermentation processes caused by various micro-organisms. During such processes the carbohydrates are split into lower-grade or directly harmful products. Organic substance is also lost in these processes as a result of the splitting off of CO_2.

3. By the breaking down of proteins, whereby the amino acids are partially totally decomposed with the formation of NH_3. These decomposition processes are also caused by micro-organisms.

Our object when we began to take up the problem of the preservation of fodder was to prevent these three processes in such a way as not to make the fodder physiologically unsuitable.

At the beginning of the 1920's we had examined the decomposition of proteins and various bacterial fermentations in our laboratory. *Inter alia*, it was apparent that these processes with the exception of lactic acid fermentation can no longer take place at a pH below 4. Since we were also able to prove that the respiration of the plant cells, too, was greatly reduced at pH 4 and totally ceased at pH 3, effective fodder preservation could be expected if an acidity below pH 4 (Fig. 3) could be brought about in the fodder sufficiently quickly. With regard to protein-rich fodder, the preservation of which is the most important from the aspect of milk production, it was generally impossible to bring about such a high acidity by means of lactic acid fermentation in the fodder, owing to the high buffer action of the fodder. Since a method based on fermentation processes is also unreliable in other

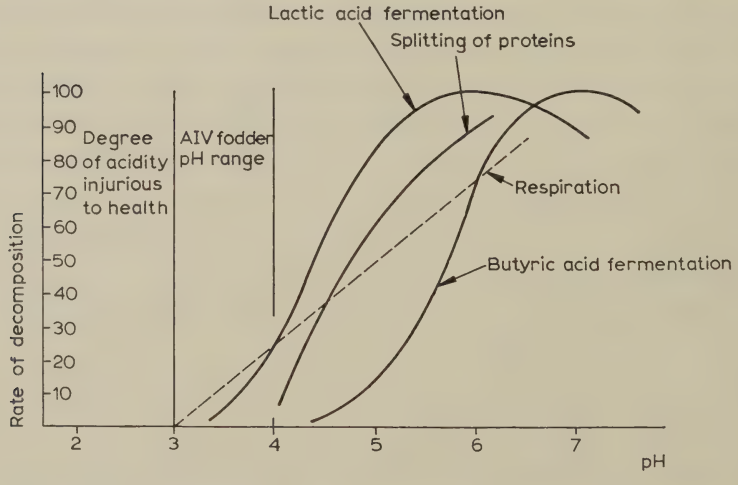


Fig. 3. Diagrammatic representation of importance of hydrogen ion concentration to various decomposition processes in compressed fresh fodder.

respects owing to the variations in the sugar content of the fodder, bacterial flora and other conditions, I considered it necessary to adopt the chemical method of controlling the fodder acidity in our search for a method of preservation reliable under all conditions.

On the laboratory scale using quantities of fodder of just a few hundred grams, we were initially able to prove the decisive feature that the acidity of the fodder was practically constant for an unlimited time if the pH of the fodder on preservation were adjusted to below 4 (pH 3–4) by means of an acid solution, and if the expressed juice formed during the compression of the fodder was allowed to run away. If, on the other hand, the quantities of acid added were such that the pH of the fodder was above pH 4, the pH of the fodder frequently rose during preservation as a result of the formation of ammonia and fermentation of the resultant lactic acid, so that an inadequate addition of acid did not prevent the harmful processes in the fodder. A pH of 4 proved to be the lowest acidity at which fodder can be reliably and effectively preserved.

Our research concerning the rate of diffusion of the added acids in the fodder showed that the penetration of the acids in the plant cells naturally

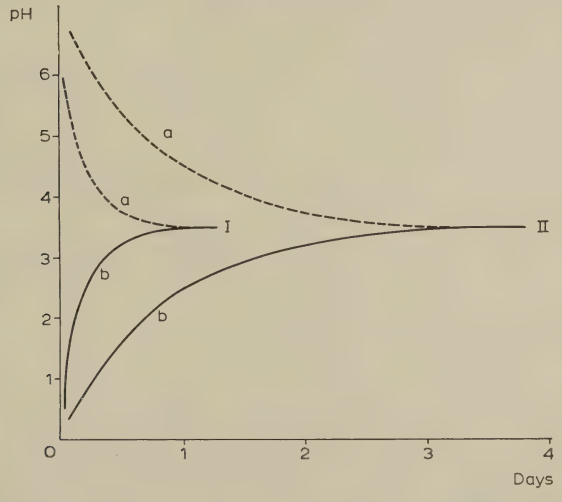


Fig. 4. Diagrammatic representation of equalization of acidity differences in fresh fodder to which sufficient acid has been added to reduce the fodder pH to 3–4.
Curves a: pH changes in the solid phase of the fodder.
Curves b: pH changes in the liquid phase of the fodder.
I: Tests with chopped fodder. *II*: Tests with unchopped fodder.

requires a certain time but is also relatively rapid with strong mineral acids Fig. 4). A rapid acidification of the fodder is very important, since in ordinary pressed fodder the splitting of both the proteins and the carbohydrates has already reached an advanced stage within two weeks (Table 2).

Quantitative experiments concerning the quantities of acids required for acidification of the fodder to a certain acidity show considerable differences for different types of fodder. The richer the plants in protein, the greater the quantities of acid required. The acidity and lime content of the soil also affects the quantities of acid. The quantities of the latter were of course also dependent upon the dry substance content of the plants and the degree of dissociation of the acids used.

Fig. 5 shows an experiment carried out with young clover (dry substance 17%) showing the effect of different acids on the degree of acidity of the fodder.

From experiments carried out with strong acids it was apparent:

1. That the quantity of strong acids required to increase the acidity of protein-rich plants to a pH of between 3 and 4 was not so great that it would be unacceptable *a priori* from the nutritional physiological or economic aspects. The quantity of weak acids required, such as lactic acid, phosphoric acid, and so on, on the other hand, was so great that their use did not seem economically worth-while at least at that time.

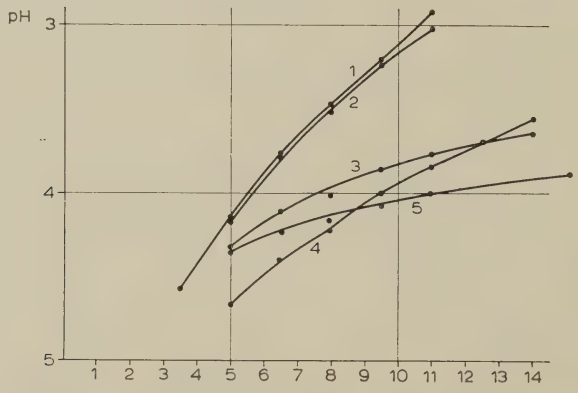


Fig. 5. Influence of various acids on the acidity of the fodder prepared from young clover. The curves show the quantities 2 *N* acid in ml per 100 g fresh clover.
Curve 1: hydrochloric acid.
Curve 2: sulphuric acid.
Curve 3: lactic acid.
Curve 4: phosphoric acid.
Curve 5: formic acid.

2. That a fodder acidified with strong acids (hydrochloric and sulphuric acid) to a pH of between 3 and 4 did not contain these acids in the free state. In the plants the cations (K, Ca, Mg, etc.) are bound by organic acids, such as oxalic, malic, fumaric, and citric acid. These plant acids are liberated by the action of mineral acid and therefore result in the acidity of the fodder. At the same time, the dissociation of the proteins is also changed, so that they also influence the final acidity of the fodder.

3. That with a fodder of a pH of between 3 and 4 we therefore did not need to take into account the influence of the free mineral acids on the state of health of the livestock. On the other hand, it was important to determine by tests with livestock whether the quantity of chloride and sulphate formed as a result of the addition of the acid might be injurious to the animals and whether the reduced base surplus might ultimately have a detrimental effect on the health of the animals.

Table 2. Preservation of chopped clover in glass cylinders, about 6.5 kg in each cylinder. The solutions are carefully mixed with the fodder, which is covered with a wooden lid and loaded with a weight of 10 kg.

	Fresh fodder	*AIV fodder*	*Ordinary pressed fodder (without additives)*	*0.7% sugar addition*	*1% NaCl addition*
Storage time 15 days (20.IX – 5.X.1934):					
pH	6.5	3.74	4.95	4.57	4.9
NH_3—N (% of total N)	0.7	2.9	13.7	10.8	14.3
Storage time 40 days (5.X – 14.XI.1934):					
pH	6.6	3.60	4.99	5.02	5.11
NH_3—N (% of total N)	0.8	1.9	14.1	15.2	16.7
CO_2-evolution (g/l kg dry substance)	—	24.5	94.8	139.9	105.3

When we had carried out the basic scientific experiments and gone over to the actual fodder preservation tests, initially only on a laboratory scale, we found that the test results agreed with the theory. It was found without exception that if the acidity of the fodder is raised to pH 3–4 from the very beginning, the harmful fermentations and splitting of ammonia from amino acids is practically completely inhibited, the formation of butyric acid is completely prevented and the evolution of carbon dioxide is reduced to a minimum.

The curves shown in Figs. 6 to 8 illustrate experiments from later years and clearly show that the splitting processes in silage are dependent upon the pH of the silage of the type of preservation method applied, and that these processes are completely controlled and reduced to a minimum only below pH 4. Above pH 4 the splitting processes increase as the pH rises and the fermentation products in the various tests will change considerably. The curves show that only pH 3–4 represents the pH range guaranteeing reliable and effective preservation.

On the basis of these results, assessment of silage quality became simple and clear: a pH analysis gives a clear idea of the fodder quality, namely fodder preserved with acid. In the case of preservation methods based on fermentations in which the extent of the decomposition processes is also dependent on how quickly the acids are formed, it is frequently necessary to determine not only the pH but also the NH_3 nitrogen expressed as a percentage of the total nitrogen.

Since the formation of ammonia in fodder acidified to pH 3–4 is minimal, we can already conclude that the preservation of the amino acids in the fodder must be excellent. Analysis of various amino acids and amino

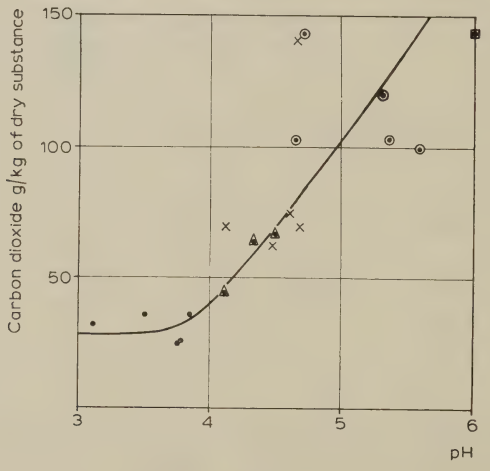


Fig. 6. The development of carbon dioxide in fodder preserved in various ways. (Five parallel tests.)

- • AIV fodder.
- × Amasil fodder (normal quantity of formic acid).
- △ Amasil-2-fodder (double the amount of formic acid).
- ⊙ Ordinary pressed fodder.
- ⊡ K.S. fodder.

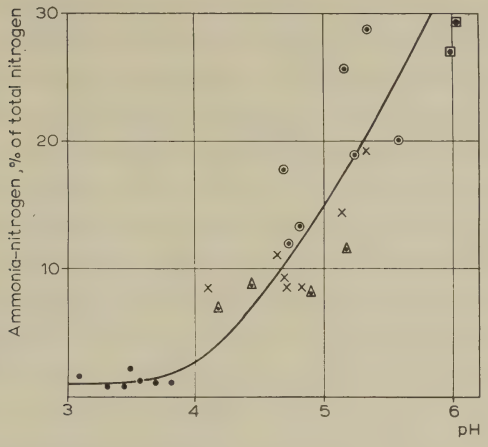


Fig. 7. Formation of ammonia in fodder preserved in various ways. (Six parallel tests.)
• AIV fodder.
× Amasil fodder (normal quantity of formic acid).
△ Amasil-2-fodder (double quantity of formic acid).
⊙ Ordinary pressed fodder.
⊡ K.S. fodder.

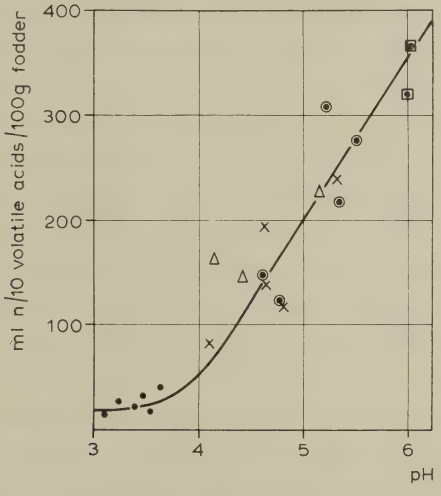


Fig. 8. Formation of volatile fatty acids in fodder preserved in various ways.
• AIV fodder.
× Amasil fodder (normal quantity of formic acid).
△ Amasil-2-fodder (double quantity of formic acid).
⊙ Ordinary pressed fodder.
⊡ K.S. fodder.

acid groups before and after preservation has shown that this is the case.

By the laboratory tests we also showed in various ways that from the preservation aspect the lactic acid fermentation in fodder acidified to pH 3–4 no longer has any appreciable significance. Although a slow lactic acid fermentation still takes place in such fodder – and there is no reason to prevent this fermentation since the nutritive value of lactic acid is practically the same as that of sugar – the quantity of lactic acid formed has no appreciable influence on the fodder acidity at such a low pH. If, for example, 1% of lactic acid is added to fodder acidified to pH 3.55 by the addition of lactic acid, the pH of the fodder drops only to 3.40. This change of the fodder acidity is insignificant from the preservation aspect. Since certain researchers still assumed at the end of the 1930's that the addition of mineral acid was important only because it ensured lactic acid fermentation, this hypothesis should be regarded as incorrect.

The new method was investigated on a large scale in 1928–1929. A particularly important question now was whether in practical fodder preservation it would be possible at all to mix the acid solution sufficiently uniformly throughout the fodder. Results in this respect were good. In these experiments the greatest attention was paid to the physiological effects of the fodder apart from certain technical questions. To begin with, we observed the cows' appetite for the artificially acidified fodder. We found that cows eagerly ate fodder prepared from clover-rich grass whose pH was between 3.4 and 3.9, in quantities equal to those that can occur in practice. If the fodder pH was below 3, the cows ate only small quantities thereof and then reluctantly. If hunger drove the cows to eat larger quantities of such fodder, their appearance quickly showed that the fodder was not wholesome (acidosis). Thus a pH of 3 could be regarded as the physiological top limit for acidity in the new process. This limit is not sharply defined but depends on the quantities of fodder eaten and the acid requirements of the preserved green fodder.

Since any harmful effects will be shown most clearly if the test animals are given large daily portions of the fodder in question, a number of cows were given 40–60 kg daily of the fodder acidified to a pH of 3.5–3.7. A test cow was slaughtered after one month's feeding and the internal organs were examined. Chemical analyses were carried out on the teeth and various tissues and also on various parts of the skeleton. The blood Ca, P, and Cl content were determined. No abnormalities were found. Similar results were obtained with two cows respectively given 4,500 and 5,600 kg of

acidified fodder during 5 and 6 months respectively, together with a daily amount of 40 g of chalk, and were slaughtered after the test period. No physiological disturbances whatever were found in many of the test cows which for months were fed with a daily portion of 23–46 kg of acidified fodder. Only the urine reaction changed during feeding from basic to acid, the CO_2 content being reduced and the NH_3 content increased. These changes can be considerably counteracted by the addition of a mixture of soda and chalk to the feeds. In the spring of 1929, when the method was put into practice, I therefore suggested the use of such an alkaline soda-chalk mixture in the case of a considerable diet of acidified fodder. In the case of a moderate diet of the fodder together with hay I did not consider it necessary to use soda. In this respect nothing has changed since then. During the past 16 years experience has dispelled any doubts as to whether fodder acidified to a pH of 3–4 really would be wholesome in practice.

To determine preservation losses in ensiling, we developed a method which makes it possible to determine these losses exactly. The method is based on the performance of tests in air-tight containers and on a quantitative observation and analysis of the gases formed and of the expressed juice running out. Since substance losses cannot occur in any other way, the method is completely reliable. *The substance losses* in fodder acidified to pH 3–4 proved to be only a few percent if the dry substance content of the raw material is so high that no pressed juice runs out of the fodder. The nutritive substance losses then do not exceed 5%. When the quantity of expressed juice rises, the nutritive substance losses also increase. If fodder having a dry substance content of 18–20% is preserved, the nutritive substance losses will not exceed 10%, as against 30–40% in ordinary silage.

The preservation method described has been used in Finland since 1929 under the name of the AIV method. In the 1930's the method was also applied in many other countries, and gradually, after much violent opposition and doubt, the theoretical principles of the method were approved more and more. It should be mentioned here that the first to confirm our experience concerning the hydrogen ion concentration significance to the preservation of fodder by their own research were Sjöberg and Köhler here in Sweden.

Before I leave the fodder preservation problem I should like to draw your attention to a central problem concerning the utilization of the AIV method and protein production in leguminous plant crops. Under the climatic conditions prevailing in our latitudes in Europe, clover-rich pastures give the richest leguminous plant crops per hectare. Farther south, blue lucerne on

suitable ground is more advantageous. Since an effective cultivation of pastures with a predominant legume stock gives the cheapest crops and the richest in protein with the minimum of work, and is also irreplaceable in the battle against weeds and in increasing the soil fertility, the endeavour should be to base milk production on this cultivation to a greater extent than previously. In this connection the AIV method offers quite new possibilities. By means of cutting time tests extending over many years in Southern Finland we have found that the best result is obtained from first- and second-year clover-rich fields – and this applies to fodder units, protein and production value of the fodder – if the fields are harvested three times per summer, the first time before the clover buds, and if all the crops are preserved by the AIV method unless the second crop is fed direct to the cattle during dry summers owing to lack of pasturage. If this procedure is adopted, one's own resources give a fodder which satisfies requirements for a fairly high milk production. This process also promotes N-fixation in comparison with the hitherto conventional practice of harvesting hay from the clover-rich fields only when it is in full bloom. As far as the labour aspect is concerned, the new system is even more profitable than the hitherto conventional.

According to one of our tests, the same clover field harvested at different times gave the following results, taking into account the losses in various methods of preservation.

Table 3. Clover-rich field, yield per hectare. First-year field 1936.

	Test Series II (cut 15/6, 24/7, 6/9)		*Test Series II (cut 1/7, 28/8)*	
	As green material	*Preserved as AIV fodder (lime)*	*As green material*	*Dried to hay (lime)*
Fodder (units/ha)	5,367	4,830	4,641	3,249
Nitrogen (kg/ha)	222	—	157	—
Digestible raw protein (g/ha)	1,055	1,002	706	529
Dry substance per fodder unit (kg)	1.27	1.3	1.37	1.7
Digestible raw protein per fodder unit (g)	197	207	152	160

On many farms in Finland 2–3 clover crops have already successfully been taken per summer for many years from clover-rich fields in connection with the AIV method. On my own test farm which I purchased in 1933 and

where this system has been applied in an extreme form since then, agriculture has been carried on without the purchase of nitrogen either in the form of nitrogenous fertilizers or protein-rich concentrates. During the past years, therefore, the entire nitrogen requirements on the farm have been covered by the use of atmospheric nitrogen. The milk production during this time has been fairly high, both per cow (about 3,500–4,000 kg of 4% milk per annum) and per hectare (over 2,000 kg of 4% milk per annum). By this practical test on a large scale, therefore, it has been proved that the use of atmospheric nitrogen in agriculture by effective legume cultivation combined with the AIV method *can* be taken to lengths such that intensive cattle farming is possible. It is in no way my intention on the basis of my experiments to try to discredit the use of nitrogenous fertilizers for non-legumes, but simply to show to what extent it is possible to dispense with the use of nitrogenous fertilizers in agriculture in cases where livestock production is the predominant feature.

The influence of the AIV method on human and animal nutrition

From the vitamin aspect, milk and milk products should be regarded as the most important foods of all. In the Scandinavian countries the use of these foods has been very great and the generally used nutrition in these countries in its best form has also been good qualitatively according to our present knowledge. A very general lack, however, has been associated with this nutrition in winter and particularly in the spring, namely a lack of vitamin A.

This is due to the fact that the vitamin A effect of milk depends entirely on the carotene content of the fodder and during the stable feeding period this content is much lower than during the summer. In connection with the work of the National Nutrition Committee of Finland we made an examination in 1936 to 1937 of the diet of the relatively poor sector of the population and fixed our attention also on the vitamin A status. We arrived at the results shown in Table 4.

Therefore, 52% of the families examined undoubtedly received insufficient vitamin A. If the milk and butter used had had the same vitamin A effect throughout the year as in the summer, the situation would have been quite different, as shown by the figures in parentheses. Only 10% would then have had too little vitamin A and then only because the quantities of milk and butter were exceptionally small.

Table 4. Vitamin A status expressed in international units in the daily food of families under examination (48 families, 318 persons) in tests carried out in spring 1936.

	Persons over 10 years at least 3000 I.U. and persons under 10 years at least 1500 I.U.	*Persons over 10 years 2000 to 3000 I.U. and persons under 10 years 1000 to 1500 I.U.*	*Persons over 10 years under 2000 I.U. and persons under 10 years under 1000 I.U.*
Percentage of persons examined	25 (73)	23 (17)	52 (10)

The figures in parentheses apply to cases where the food remains unchanged in respect of its composition but the milk and butter used had had the same vitamin A effect as in the summer.

This result illustrates the enormous significance in human nutrition of the changes in the vitamin content of milk during various seasons. This disadvantage is all the more striking since the value of milk and milk products as a vitamin source is if anything even greater during the winter and spring than during the summer. According to investigations made in our laboratory, only a small part of the carotene in carrots is resorbed in the intestinal canal, so that the importance of milk and milk products as a source of vitamin A has become all the clearer. Van Eekelen found practically no resorption of the carotene of carrots in man.

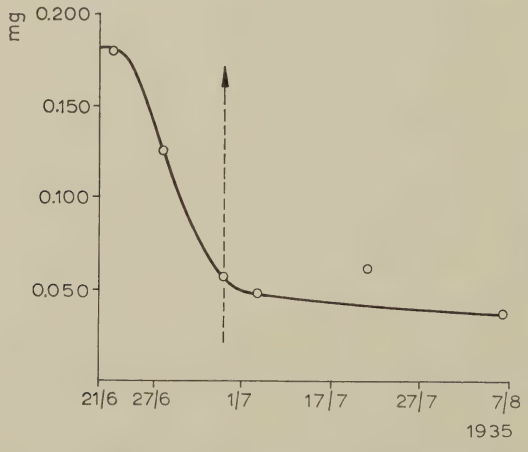


Fig. 9. Carotene content of red clover.
(The vertical broken line shows the beginning of flowering. Carotene expressed per 1 g of dry substance.)

At the beginning of the 1930's we began to investigate the formation and importance of vitamins in plants and the preservation of the vitamins in silage. From the experiments relating to the formation of the vitamins, it was apparent that at least carotene and vitamin C, which were the object of our attention, are bound up with the growth of the plants to such a degree that I considered there to be reason for assuming that the said vitamins should be regarded as plant hormones. All the factors which promoted the plant growth, such as the application of fertilizers and optimum acidity of the soil, also increased the plant carotene and vitamin C content. The observation that the vitamin content of grass plants and legumes dropped rapidly when the plant approached the flowering stage (Fig. 9), became particularly significant. This observation showed that the quantity of vitamin A received by livestock in their feed varies enormously depending upon the stage of development of the fodder plants.

The experiments whereby we examined the effect of crystalline vitamin C on plant growth using the sterile cultivation system clarified the importance of vitamins to plants. The test results given by Synnöve v. Hausen in her doctorate thesis positively proved the absolute necessity of vitamin C as a growth factor for plants. When she removed the cotyledons from peas at a suitable growth stage – the cotyledons contain practically all the vitamin C formed during germination of the seed – the pea could no longer grow. By the addition of vitamin C to the culture solution of such plants the pea could be made to grow again. This important observation in conjunction with our previous research concerning the ability of plants to assimilate organic compounds of the soil shows that carbon compounds present in the soil may also have great significance for plant growth.

Our research concerning the clarification of the preservation of the vitamins in silage showed that the AIV method is absolutely ideal in preservation of the vitamins. Carotene is preserved practically quantitatively, and the vitamin C preservation – in comparison with other methods of preservation – is excellent, and the same also appears to apply to other vitamins. When hay is dried and stored in barns, on the other hand, the carotene deteriorates to a great extent. During spring the carotene content drops, according to circumstances, to 10 to 25% of the original value. The vitamin C disappears practically quantitatively during drying. These changes are important to the animals' health and the utilization of the feed, at least insofar as concerns carotene. In tests carried out with various livestock the AIV fodder proved extremely important if the other feeds were deficient in carotene.

This research fully explained the enormous drop in the vitamin A effect of milk during the stable feeding period. As an immediate result of the low carotene content of hay, during the stable feeding period the cow receives in its feed only a fraction of the quantity of carotene than during pasturage. The low carotene content of hay is due partially to the fact that grass crops when harvested as hay are not cut until the beginning of flowering or in full flower, and partly because of oxidation of the carotene during drying and storage of the hay.

If the AIV method is used, the carotene is retained practically completely. Since the method also includes cutting the grass at an early stage of growth, the AIV fodder corresponds largely to the summer feed of the cattle. If a cow receives daily about 30 kg of AIV fodder prepared from young clover-rich grass during the stable feeding period, the vitamin A effect of the milk is practically the same as during summer pasturage. Table 5 shows the research result in 1937 to illustrate the vitamin A effect of milk of various breeds of cow under different feeds.

A considerable increase in the vitamin A effect of milk as a result of AIV feeding has been found by many other researchers, such as Peterson, Brouwer, etc. both in the U.S.A. and Europe. The value of the vitamin A effect of milk produced during stable feeding as compared with pasturage milk depends partly on the quantity of AIV fodder given and partly on the raw material from which the fodder was prepared.

The AIV fodder has a favourable effect not only on the milk vitamin A content but also on the quantity of vitamin B_2. We have found that if cattle are given a plentiful supply of AIV fodder throughout the year, the lactoflavin content is 25–30% higher than with ordinary stable feeding. Since vitamin B_2 is formed in the rumen and the cow therefore can manage with a feed free from vitamin B_2, the influence of the feed on the lactoflavin content of milk is not immediately clear.

Elvehjem, Peterson, and colleagues in the U.S.A. have shown that the milk produced on pasturage or with AIV fodder contains much more so-called « grass sap factor » than ordinary winter milk. The effect of this factor can be demonstrated if rats are fed solely with milk supplemented by Cu, Mn, and Fe salts.

The AIV fodder is rich in vitamin C which again is practically completely lacking in hay. The vitamin C content of milk is not affected by the fodder, but a fodder rich in vitamin C does improve the quality of the milk. If the fodder does not contain sufficient vitamin C, the milk acquires a tallowy

taste which is quite usual in milk resulting from hay and concentrated feeds. This defect never occurs in pasturage or AIV milk. It is not known why the oxidase enzymes increase or why the factors which inhibit oxidation decrease in milk when the fodder is deficient in vitamin C. According to the latest American research, the substances which cause the tallowy taste reduce the utilization of vitamin A and are detrimental to health.

Table 5. Vitamin A effect of butterfat. (Expressed in international units* per 100 g of butterfat.)

		Stable feeding				*Pasturage feeding*	
		Without AIV fodder		*With plentiful AIV fodder*			
Farm number	*Breed of cow*	*Total vitamin A effect (I.U.)*	*Proportion of carotene in vitamin A effect (%)*	*Total vitamin A effect (I.U.)*	*Proportion of carotene in vitamin A effect (%)*	*Total vitamin A effect (I.U.)*	*Proportion of carotene in vitamin A effect (%)*
1–2	Ay	1,833	20	—	—	3,783	21
14–16	Ay	—	—	4,000	22	4,450	26
3–6	LSK	2,083	20	—	—	4,100	24
18–19	LSK	—	—	3,583	28	—	—
7–8	ISK	2,000	20	—	—	4,234	25
17	ISK	—	—	4,050	26	5,099	26
9–13	PSK	2,817	15	—	—	4,249	23

* 1 vitamin A international unit = 0.6 γ carotene = 0.3 γ vitamin A.

The above differences between milk produced with different feeds show the many-sided influence of cattle feed on the biocatalysts and nutritive value of milk.

Research to increase the quality of milk products

The use of milk for human nutrition is also essentially associated with the processing of milk to form various products. It is important that these products are of the highest possible quality both in respect of taste and nutritive physiological properties, and that they keep well. As already mentioned, the

oxidation of fat in milk and milk products also results in deterioration of their nutritive physiological properties.

In this connection I will just briefly deal with one aspect of our research which has been of great practical importance, namely work in connection with the keeping-quality of butter, which clearly shows the decisive influence of the hydrogen-ion concentration on the oxidation phenomena in butter. Butter with the best flavour is prepared from sour milk. During the souring process a pleasant aroma develops, which originates mainly from diacetyl. Although such high-quality butter is prepared with the maximum care, all possible infection being avoided, it cannot be stored for any appreciable length of time. At a temperature above 0° it frequently acquires an oily or fishy taste or even a tallowy taste after a few weeks, and in cold storage after 1–3 months. These defects reduce the value of the stored butter considerably and it is difficult normally to market such butter. In 1925 I was successful in finding a way of preventing these defects. It was in fact found that the oily or fishy taste which is probably due to oxidation products of lecithin, occurs in butter if the pH of the latter drops below 6. The lower the pH of the butter, the more quickly this defect occurs. Heavy metals, more particularly copper, catalyze this reaction strongly, but the butter still contains so much heavy metals that the oily taste occurs sooner or later. At a pH above 6 the oily or fishy taste never occurs, so that defects associated with this

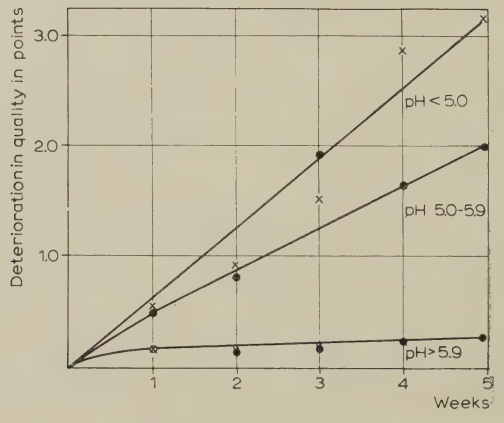


Fig. 10. Deterioration of quality in butter samples whose pH was below 5, between 5.0 and 5.9, and above 5.9. In the latter the pH in the butter was increased by the addition of disodium phosphate. The deterioration of the butter quality is expressed in points counting from the number of points the butter had when fresh. (Storage time is expressed in weeks.)

group can be prevented by adjusting the pH of the butter to above 6 (Fig. 10). For this reason I proposed the use of a salt in the preparation of butter, in which salt was mixed a suitable quantity of basic substances, for example disodium phosphate and anhydrous soda. In this way it was possible to adjust the butter acidity to pH 6–7, which guaranteed its storability. This simple method was of decisive importance in the storage of butter and the quality thereof. The discovery also made it possible to separate the bacteriological butter defects from the chemical and it has therefore resulted in ready controllability of butter quality.

Ladies and gentlemen. I have now come to the end of my lecture. We have followed the fixation of atmospheric nitrogen and the formation of amino acids in the root nodules of legumes, the production of protein-rich legume crops, the preservation of them to form a high-grade animal feed, and the production of physiologically high-grade milk adapted to the improvement of human nutrition. These questions represent widely different problems within biochemical research but are bound up with one another organically, and form a whole from the practical aspect.

I consider myself fortunate in having had the opportunity of working in this interesting field and in having advanced development somewhat. The Nobel Prize will both directly and indirectly increase our research facilities, and the Swedish Academy of Sciences will therefore be promoting biochemical research in my country, and for this I should like to express my deeply felt gratitude.

Biography

Artturi Ilmari Virtanen was born in Helsinki on the 15th of January, 1895, as the son of Kaarlo Virtanen and Serafiina Isotalo. He was educated at the Classical Lyceum at Viipuri, Finland. After finishing school, he studied chemistry, biology, and physics at the University of Helsinki, where he took his M.Sc. in 1916 and obtained his D.Sc. in 1919. Subsequently he studied physical chemistry in Zurich in 1920 under G. Wiegner, bacteriology in Stockholm in 1921 under Chr. Barthel, and enzymology in Stockholm during 1923–1924 under H. von Euler. Since 1923, his interest turned to biochemistry.

He was first-assistant of the Central Laboratory of Industries at Helsinki during 1916–1917, and chemist in the Laboratory of Valio, Finnish Cooperative Dairies' Association, during 1919–1920. In 1921 he became Director of this laboratory, and in 1931 of the Biochemical Research Institute at Helsinki. After having been docent in chemistry at the University of Helsinki since 1924, he was appointed Professor of Biochemistry at the Finland Institute of Technology at Helsinki in 1931, and at the University of Helsinki in 1939. Since 1948 he has been member and President of the State Academy of Science and Arts in Finland.

Professor Virtanen is a member of the Finnish, Norwegian, Swedish, Flemish, Bavarian, and Pontifical Academies of Science, and of the Swedish and Danish Academies of Engineering Sciences. He is an honorary member of learned societies in Finland, Sweden, Austria, Edinburgh, and the U.S.A., and holds honorary degrees of the Universities of Lund, Paris, Giessen, and Helsinki, the Royal Technical College at Stockholm, and the Finland Institute of Technology. Numerous medals and other distinctions have been conferred upon him from Sweden, Finland, Belgium, and Italy.

Virtanen established the indispensability of cozymase in lactic and propionic acid fermentations, as well as the phosphorylation of sugar (1924). In these works the similarity of different fermentation processes as to the first stages in the decomposition of sugar became apparent. Together with his collaborators he continued the fermentation experiments, special atten-

tion being paid to the mechanism of different bacterial fermentations. The fermentation of dioxyaceton to glycerol and glyceric acid in the presence of phosphates by the effect of *Coli* bacteria (1929) was the first sugar fermentation which was elucidated chemically from beginning to end. In this work attention was also paid to the adaptive formation of enzymes, which phenomenon his collaborator H. Karström treated in great detail in his doctor's thesis (constitutive and adaptive enzymes). The phenomenon of adaptation, and in connection with it the uptake of nutrients by cells, is still subjected to investigations in his laboratory. The concept that almost all proteins in bacterial cells are enzyme proteins led to investigations on the relation between the protein content and enzymic activity of cells.

Since 1925, the biological nitrogen fixation which takes place in the root nodules of leguminous plants has been subjected to many-sided investigations in his laboratory. The importance of the red pigment, leghaemoglobin, in active root nodules for the fixation of nitrogen was proved.

The formation of vitamins in plants, as well as the ability of plants to utilize organic nitrogen compounds as their nitrogen source, have been treated in many publications from his laboratory.

Since the end of the 1940's, the chemical composition of higher plants has been given special attention in his laboratory. A large number of new amino acids have been isolated from different plants, and have been characterized chemically. Numerous organic sulphur compounds, which may be of importance for the nutrition of man and domestic animals, have also been isolated from vegetables and fodder plants.

The application of biochemistry to agriculture and the dairy industry belongs to the practical activities of his laboratory. Among works performed in this field are the creation of a theoretical basis for the preservation of fresh fodder and the development of a practical method on this basis (the AIV method), with the aim to promote an effective utilization of protein-rich crops, and to produce milk of the same vitamin content in winter as that produced on summer pastures. Investigations aiming at the improvement of the quality of dairy products also have to be mentioned as belonging to the field of applied research.

Virtanen married Lilja Moisio in 1920; they have two sons, Kaarlo and Olavi.

Chemistry 1946

JAMES BATCHELLER SUMNER

«for his discovery that enzymes can be crystallized»

JOHN HOWARD NORTHROP

WENDELL MEREDITH STANLEY

«for their preparation of enzymes and virus proteins in a pure form»

Chemistry 1946

Presentation Speech by Professor A. Tiselius, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesty, Royal Highnesses, Ladies and Gentlemen.

In 1897 Eduard Buchner, the German research worker, discovered that sugar can be made to ferment, not only with ordinary yeast, but also with the help of the expressed juices of yeast which contain none of the cells of the *Saccharomyces*. The discovery was considered so important that in 1907 Buchner was awarded the Nobel Prize for Chemistry.

Why was this apparently somewhat trivial experiment considered to be of such significance? The answer to this question is self-evident, if the development within the research work directed on the elucidation of the chemical nature of the vital processes is followed. Here, as in other fields of research, progress has taken place step by step, and the conquest of new fields has often been very laborious. But there, more than in most fields, a tendency has showed itself to consider the unexplained as inexplicable – which is actually not strange where problems of life and the vital processes are concerned. Thus ordinary yeast consists of living cells, and fermentation was considered by the majority of research workers – among them Pasteur – to be a manifestation of life, i.e. to be inextricably associated with the vital processes in these cells. Buchner's discovery showed that this was not the case. It may be said that thereby, at a blow, an important class of vital processes was removed from the cells into the chemists' laboratories, to be studied there by the chemists' methods. It proved, too, that, apart from fermentation, combustion and respiration, the splitting up of protein substances, fats and carbohydrates, and many other similar reactions which characterise the living cell, could be imitated in the test tube without any cooperation at all from the cells, and that on the whole the same laws held for these reactions as for ordinary chemical processes. But – and this is a very important reservation – this was only possible if extracts or expressed juices of such cells were added to the solution in the test tube. It was then natural to assume that these cell juices or cell extracts contained some substance which had the capacity of initiating and maintaining the reactions and guiding them into the paths they follow in the cell. These unknown active substances were called enzymes or

ferments, and the investigation of their effects became one of the principal problems of chemistry during the first decades of this century, which for the rest it still is.

The important question of the nature of the enzymes remained unsolved, however, in spite of the energetic efforts of the research workers. It is manifestly a question of substances of complicated structures, which are present in such extremely small amounts that they, so to speak, slip through the fingers when one tries to grasp them. It is really remarkable to see how far it was possible to get in the study of the effects of the enzymes and the course of the enzymatic reactions, without knowing anything definite about the nature of these very active substances, nay, even without even being quite clear that they were substances which could be isolated in the pure form at all.

In 1926, however, in connection with his studies of a special enzyme «urease», James B. Sumner of Cornell University, Ithaca, U.S.A. succeeded in producing crystals which exhibited strikingly great activity. The basic material was the bean of a South American plant, *Canavalia ensiformis*, in America called the «jack bean», and the crystals had an activity that was about 700 times as great as that of bean flour. What was still more important was that it was possible to dissolve the substance and re-crystallize it several times without its activity being affected. The crystals proved to consist of a protein substance. Sumner expressed the opinion that in reality this protein substance *was* the pure enzyme.

As is so often the case with important discoveries, this result will probably to a certain degree have «been in the air», in that at the time it had been assumed in many quarters that the enzymes were protein substances of quite a special nature. On the other hand, Willstätter, the German chemist and Nobel Prize winner, had carried out far-reaching purifying experiments with enzymes and had arrived at results which caused him to doubt whether it was a question of protein substances or carbohydrates at all. We know now that this was due to the fact that Willstätter's purifying methods yielded solutions which were all too weak for it to be possible for chemical reactions to give a definite result.

For the chemist crystallization is the final goal in the preparation of a substance in pure form. Even though crystallizability is not such a reliable criterion of purity in the case of protein substances as in that of simpler substances, nevertheless Sumner's results have now been accepted as verified and thus also accepted as the pioneer work which first convinced research workers that the enzymes are substances which can be purified and isolated in tangible

quantities. Thereby the foundation was laid for a more detailed penetration of the chemical nature of these substances, on which an understanding of the reactions taking place in living cells must finally depend.

Sumner's pioneer work was not immediately followed by similar work in other quarters, which might perhaps have been expected. About three years after Sumner's work had been published, however, Dr John Northrop of the Rockefeller Institute at Princeton began to work on the purification of the protein-splitting enzymes met with in the digestive apparatus and gradually succeeded in obtaining a number of them in crystallized form, e.g. the pepsin met with in the gastric juice and the trypsin and chymotrypsin in the pancreas. Northrop and his collaborators, among whom should be mentioned in the first place Kunitz, also made extremely comprehensive studies of the homogeneity and purity of these purified enzymes, and in that connection gave further proof of their nature as protein substances. Exceedingly interesting results were attained also in the isolation of some protein substances which appeared to be the mother substances of these enzymes. On the whole Northrop used his purified material for detailed chemical studies to a greater extent than did Sumner, and his contributions in the matter of working out the most satisfactory conditions for the crystallization of enzymes have been of the greatest importance for subsequent research workers.

This year's third Nobel Prize winner in Chemistry, Dr Wendell Stanley, first worked at the Rockefeller Institute in New York but moved in 1932 to the department of that Institute at Princeton. The problem which attracted his attention, namely the chemical nature of the viruses, was to a certain degree analogous to the problem of the enzyme just mentioned. As is well known, viruses are contagia which give rise to a large number of the best known illnesses in man, animals and plants, e.g. smallpox, infantile paralysis, influenza, foot-and-mouth disease, mosaic disease (on tobacco plants), etc. The virus particles are invisible in the microscope, and when Stanley began his work, they could only be identified by the symptoms of disease which they occasioned. Thus the problem was more difficult, inasmuch as the effect of the virus could not be as easily measured as that of an enzyme, where an exactly known chemical reaction can be employed. Stanley first tried to show the protein nature of viruses by studying how the virus of the tobacco mosaic disease was attacked by protein-splitting enzymes, but in 1934 he passed on to attempting to purify that virus by methods similar to those which Sumner and Northrop had employed so successfully for enzymes. In 1945, by using large quantities of infected tobacco leaves, he did succeed in

producing small amounts of crystals which were extremely active, and which, after detailed investigation, proved to be the bearers of the virus's activity. Here, too, it was a matter of active protein substances. Subsequently it has been proved that nucleic acid also forms an important constituent of the latter.

It seems as though Stanley's discovery may take us another long step forward along the road towards a closer understanding of the chemical nature of the vital processes, for apart from the fact that in extremely small quantities they can give rise to diseases, the virus substances, like the bacteria, have the capacity to reproduce themselves. It was remarkable enough when Buchner found that certain of the functions of the living cell can be separated out from it and are to be found in the expressed juice, but it appears still more remarkable that the capacity to reproduce – this unique characteristic of life – can also be exhibited by certain molecules, thus by dead substances. It must be borne in mind, however, that, as far as we know now, this capacity is only possessed by the virus molecule when it is in contact with the living cell, and that probably the latter is materially responsible for the reproduction of the virus substance.

Investigations both by Stanley and by other research workers show that many kinds of viruses, e.g. the smallpox virus, are considerably more complicated in structure. It is conceivable that the «molecular virus» which Stanley isolated represents the simplest type in a long series of different kinds of viruses which gradually approach the living bacteria. An extraordinarily fascinating field is hereby opened up to research workers, and it is not improbable that development will lead to a closer scrutiny of the border-line between living and dead matter.

Even among scientists we sometimes hear the assumption expressed that the innermost secrets of the vital processes will always be hidden from us, that there is a wall through which we cannot penetrate. Today we do not know whether that be correct, but we know that this wall – if there is one – is considerably farther away than one had dared to believe earlier. That this is so is to an appreciable degree the result of the discoveries which have been rewarded with the 1946 Nobel Prize for Chemistry.

Doctor James Sumner. Your discovery of the possibility of crystallizing an enzyme was the first convincing proof that enzymes are proteins. It thus became possible, for the first time, to attack the problem of the chemical nature of these substances, so important in chemistry, biology and medicine.

As is often the case with pioneer work, your results were not immediately accepted everywhere, but today they form a foundation for and have pointed the way to some of the most important work in biochemistry.

Doctor John Northrop. You and your collaborators have developed the crystallization of enzymes and other active proteins into an art, of which you are the masters. The conditions for successful work in this field were explored by you, and in the course of that work interesting relationships between enzymes and related proteins were discovered, which may ultimately afford a clue to a fuller understanding of the mode of action of these substances.

Doctor Wendell Stanley. We owe to you one of the most striking discoveries in modern chemistry and biology. The demonstration of the fact that a virus can be crystallized in the same way as many proteins and enzymes, and that it actually *is* a protein, at once opened up an almost unlimited field of research with fascinating possibilities. You have not only thrown open the portals to this domain, but you are yourself successfully exploring its possibilities, and rich fruits have already been harvested, thanks to your own work and that of your school.

Gentlemen. The fundamental problems which you have attacked and solved with such remarkable success are closely related, and the methods used have much in common. The more recent achievements have added to the significance of the earlier advances in this field. Your work and your discoveries deserve the gratitude of mankind. The award to you of the Nobel Prize in Chemistry for 1946 is an expression of this gratitude.

Doctor James Sumner, Doctor John Northrop, Doctor Wendell Stanley. With the warmest congratulations of the Academy I now ask you to receive your awards from the hands of His Majesty the King.

James B. Sumner

The chemical nature of enzymes

Nobel Lecture, December 12, 1946

Professor Westgren, Ladies and Gentlemen.

Why was it difficult to isolate an enzyme? Here I employ the word «isolate» as meaning preparing in pure condition. It does not seem difficult to isolate and crystallize an enzyme now, but it was difficult 20 years ago. The reasons were several. One was the inertia of men's ideas. Another reason was the influence exerted by Willstätter and his school, who held that enzymes were neither lipids, carbohydrates nor proteins and who believed enzymes to exist in excessively low concentrations in plants and animals. The misconception that colloidal substances had a chemistry different from that of crystalloids was another stumbling block. A yet more important reason why enzymes could not be isolated readily was because work with these substances requires a special technique. The enzyme is present in relatively low concentration in the raw material. It is often highly unstable. The raw material nearly always contains natural protectors which are left behind during the purification processes. In the absence of these natural protectors the enzyme may become destroyed by traces of heavy metals, by oxidation, by unfavorable pH, or even by autolysis.

I wish to tell next why I decided in 1917 to attempt to isolate an enzyme. At that time I had little time for research, not much apparatus, research money, or assistance. I desired to accomplish something of real importance. In other words, I decided to take a « long shot ». A number of persons advised me that my attempt to isolate an enzyme was foolish, but this advice made me feel all the more certain that if successful the quest would be worthwile.

The reasons why I chose to work with urease were several. I had been working with urease as a reagent for the estimation of urea in muscle, blood and urine. This urease was prepared from soybean meal. In 1916 Mateer and Marshall found that the jack bean, *Canavalia ensiformis*, contained about 16-fold more urease than the soy bean. The jack bean appeared to me to be extraordinarily rich in urease and I could see no reason why this enzyme could not be isolated in pure form and characterized chemically. Claude Bernard has said that success or failure may depend upon the lucky choice of

some reagent or raw material. Willstätter was unfortunate in his choice of saccharase as an enzyme to isolate. I was fortunate in choosing urease.

I hoped that urease would turn out to be a globulin, since globulins are usually, if not always, present in beans and seeds, and since globulins are easy to precipitate by dialysis. Other reasons for choosing urease as the enzyme to isolate were because this enzyme can be estimated quantitatively very accurately and readily and because urease could be expected to be one enzyme, rather than a mixture of enzymes, acting as it does on such a simple substrate as urea.

I started trying to isolate urease in the fall of 1917, having been occupied previously with analytical methods. At this time our laboratory contained no adequate apparatus for grinding jack beans. We first used a coffee mill and then ground the coarse material with a mortar and pestle. Years later we constructed a mill which was run by an electric motor and which employed a bolting device. However, in the meanwhile we usually used commercial jack bean meal. This commercial meal was not always satisfactory.

The jack bean is a miniature world in itself and contains all of the elements required for life, growth and reproduction. I decided to isolate and characterize as many as possible of the chemical compounds present in the bean. I found various minerals, proteins, carbohydrates, lipids, extractives, pigments and enzymes to be present. Particular attention was paid to the proteins of the jack bean, since I expected to find that urease was one of these. I isolated two globulins in crystalline form and named these concanavalin A and concanavalin B. A third globulin which I called « canavalin » separated as spheroids upon dialysis. Years later Howell and I were able to crystallize this, after a preliminary digestion with trypsin. Many years later we discovered that concanavalin A is a hemagglutinin for the red cells of certain animal species as well as an excellent precipitant for certain polysaccharides. A lipid fraction of the jack bean was observed to function as a thromboplastic agent.

In attempting to concentrate and purify urease I employed fractional precipitation with alcohol, acetone and other organic solvents. Fractional precipitation with ammonium sulfate, magnesium sulfate and other neutral salts was tried. I tested a large number of salts of heavy metals as precipitants. I employed a very large number of reagents as adsorbents. This work covered many years. At times I grew discouraged and temporarily abandoned the quest, but always returned to it again.

At first we used to extract urease from jack bean meal with water. These aqueous extracts were viscous and therefore very difficult to filter. Glycerol

extracts were even more bothersome. I learned that Folin used 30 per cent alcoholic extracts of jack bean meal as a source of urease for analytical purposes. It was found that extraction with 30 per cent alcohol was of distinct advantage, inasmuch as this solvent dissolved most of the urease but failed to dissolve a rather large quantity of the other proteins. Hence a considerable purification was achieved through the use of this solvent. The alcoholic extracts filtered very rapidly, leaving the undissolved material behind on the filter paper. The only disadvantage of 30 per cent alcohol lay in the slow inactivating action of this solvent upon the urease. However, if kept at low temperatures there was no inactivation of the enzyme.

When kept at low temperatures 30 per cent alcoholic extracts of jack bean meal formed precipitates. These precipitates contained practically all of the urease, together with concanavalin A, concanavalin B and other proteins. At this time we had no ice chest in our laboratory and we used to place cylinders of 30 per cent alcoholic extracts on our window ledges and pray for cold weather.

It seemed to me of interest to employ dilute acetone instead of 30 per cent alcohol and to see whether this substitution would result in any improvement in the method of purification. Accordingly I diluted 316 ml of pure acetone to 1000 ml and used this as the means of extracting the urease. I

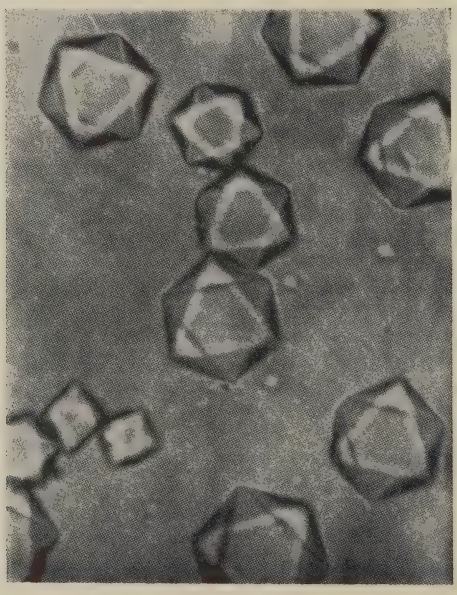


Fig. 1. Urease crystals.

routinely employed this dilution of acetone, since I had been preparing 30 per cent alcohol through diluting 95 per cent alcohol in this manner. The acetone extract was chilled in our newly acquired ice chest overnight. The next morning I examined the filtrate. It contained practically no precipitate, thus differing from alcoholic filtrates. However, upon observing a drop of the liquid under the microscope it was seen to contain many tiny crystals. These were of a shape that I had never observed previously. I centrifuged off some of the crystals and observed that they dissolved readily in water. I then tested this water solution. It gave tests for protein and possessed a very high urease activity. I then telephoned to my wife, «I have crystallized the first enzyme».

Now, I should like to tell this audience what enzyme crystals look like. (See Figs. 1 and 2.) This description applies also to proteins, since enzymes are proteins. Enzyme crystals are highly refractive and nearly always microscopic, since these compounds, being of high molecular weight, diffuse relatively slowly and therefore crystallize slowly. Enzyme crystals belong nearly always to the isometric or hexagonal systems. Enzymes may separate from solution as spheroids. This indicates a tendency to crystallize, as Dr. Northrop can tell you from his experience. Spheroids are nearer the crystalline state than purely amorphous material, such for example, as casein which has been

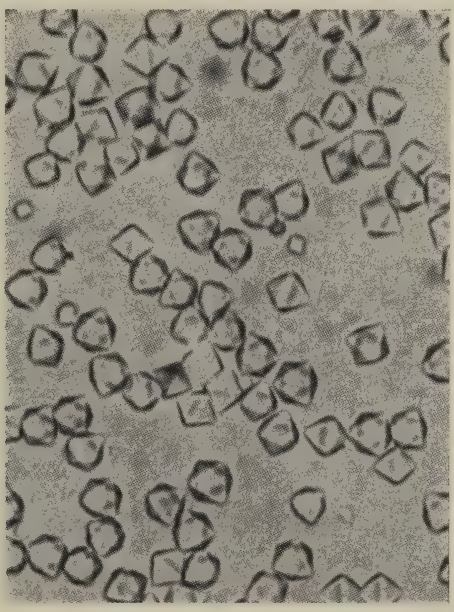


Fig. 2. Urease crystals.

precipitated from milk by the addition of acetic acid. Spheroids have sometimes been found by us to be aggregations of many needles either in parallel or concentrically arranged.

Having convinced myself that I had really isolated urease in crystalline form, I read a paper on this matter at Clifton Springs, New York and published an article in the August number of the Journal of Biological Chemistry for the year 1926. But now a difficulty arose. The commercial jack bean meal which we had been using suffered a decline in quality and we could obtain no urease crystals from it unless we added a small amount of acetic acid to the alcoholic filtrates. Even then, the yield of crystals was low. Analyses showed that the recent samples of jack bean meal contained only about one-half as much urease as the earlier samples. Accordingly attempts were made to obtain satisfactory meal, or satisfactory jack beans. We grew jack beans in one of the Cornell greenhouses. The beans grew poorly and the yield was less than the number of beans planted. We obtained jack beans from Texas, Guatemala and Honduras, but these were low in urease. I happened to meet a plant physiologist, Dr. Albert Muller, who said that he would grow me some jack beans at Mayaguez, Porto Rico. I gave him about a kilo of jack beans rich in urease, the last I had left. Some seven months later about a bushel of beans arrived from Porto Rico. These beans were rich in urease. The finely ground meal gave a high yield of urease crystals. Later on, we were able to obtain excellent jack beans from an Arkansas farmer. He has supplied us with jack beans ever since.

I wish to speak now about proofs of the identity of the crystals with urease. In cases of this sort one piece of experimental evidence is not sufficient to constitute a valid and satisfactory proof; one must submit many pieces of evidence. At this time I had no access to the ultra-centrifuge of Svedberg nor to the electrophoresis apparatus of Tiselius. However, I was able to offer evidence of another sort, as is shown below:

1. When the crystals separated there occurred a great increase in purity, namely from 700 to 1400-fold. Using other methods the increase in purity observed was very much less than this and at times there even occurred a decrease in purity.
2. When the distribution of urease was followed quantitatively it was found that of the urease passing into the filtrate as much as 40 per cent or more separated with the crystals.
3. Recrystallization increased the purity of the urease.
4. Adding traces of poisons, such as mercuric chloride or formaldehyde, to

jack-bean meal inactivated the urease and, at the same time, prevented the appearance of the crystals.

In obtaining the crystals I felt much the same as a person does who is trying vainly to place in position a piece of a machine. Suddenly the piece slides in as if covered with butter. One knows that it is now where it belongs.

During later work in crystalline urease I was fortunate to have in our laboratory a number of excellent men. These were Doctors Hand, Howell, Kirk, Poland and Dounce. We found that trypsin neither digested nor inactivated urease. Since trypsin is a proteolytic enzyme of second attack, it does not digest certain native proteins readily. Some proteins that are digested with great difficulty are hemoglobin, ovalbumin and the serum proteins. After urease had been denatured by acid or by heating it was found to be very readily digested by trypsin.

Pepsin acts best in a strongly acid medium and strong acid rapidly destroys urease. However, at pH 4.3 we found that urease was so slowly destroyed that it was possible to demonstrate a parallel digestion and inactivation by pepsin. In place of pepsin it was possible to use papain-cysteine.

In our laboratory Dr. J. Stanley Kirk was able to immunize rabbits to crystalline urease. Kirk started by giving rabbits as little as 0.03 mg of crystalline urease intraperitoneally. This dose was given twice weekly and was finally increased to 1000 lethal doses. The immunized rabbits contained antiurease in their blood serum. This antiurease could be purified by precipitating it by adding urease, washing the urease-antiurease precipitate, decomposing the complex with 0.05 *N* hydrochloric acid, bringing to pH 5.0 and centrifuging down the denatured urease. The antiurease was not harmed by this treatment and could be employed as an excellent precipitant in testing for urease. It gave a visible precipitate with solutions of urease diluted 1 to 500,000. However, urease allowed to stand for a few seconds with 0.05 *N* hydrochloric acid and then neutralized gave no precipitate with antiurease; neither did it possess any urease activity.

Northrop has made good use of physical methods to demonstrate the identity of pepsin with his pepsin crystals. While we have not employed such methods, Kubowitz and Haas, working in Warburg's laboratory have demonstrated that ultraviolet light is absorbed by highly dilute solutions of crystalline urease and that exactly the same wavelengths that are absorbed are those which destroy urease.

In 1930 Northrop of the Rockefeller Institute obtained pepsin in crystalline form. A short time later Northrop and Kunitz obtained crystalline tryp-

sin, crystalline chymotrypsin and also the zymogens of these enzymes in crystalline form. This monumental work was of very great help in bringing the scientific world to admit that enzymes can be isolated in pure and crystalline condition. In this connection I wish to note that Professor von Euler aided me greatly when I worked on urease in his laboratory at Stockholm's Högskola in 1929 and that I received valuable help in 1937 while working in the laboratory of Professor The Svedberg at the University of Uppsala.

The announcement of the crystallization of urease and pepsin was not accepted by some biological chemists. In Germany students of Willstätter attempted to show that our crystalline proteins were merely carriers of the enzymes. It suffices to say that these attempts to disprove our work failed, as they were bound to fail, since Northrop and I were right and since our evidence was unassailable.

To date about thirty enzymes have been obtained in crystalline and in presumably pure condition. Certain enzymes are colored, due to being combinations of specific proteins with such prosthetic groups as riboflavin phosphate, or hematin. Theorell has crystallized the yellow enzyme of Warburg and Christian and, remarkably enough, has been able to split the protein from the riboflavin phosphate and later to reunite these two components. He has shown conclusively that the union of these components proceeds stoichiometrically.

The enzymes catalase and peroxidase are compounds of protein with hematin and these two enzymes possess characteristic absorption bands. This property has greatly facilitated the crystallization of these two enzymes. In addition, it should be noted that these two enzymes have been shown to form compounds with hydrogen sulfide, sodium fluoride, sodium azide, etc.

All enzymes are proteins but not all proteins are enzymes. Many, if not all enzymes can be crystallized. The oxidizing enzymes all appear to be conjugated, or compound, proteins, while the hydrolytic enzymes, are, as far as we can tell, without prosthetic groups. However even hydrolytic enzymes must have reactive groups. Every enzyme requires a specific method for its purification. Our present methods of purifying both enzymes and proteins are crude and unsatisfactory for the most part. It is fairly certain that better methods will be discovered in the near future. One can purify an enzyme either by precipitating the impurities or else by precipitating the enzyme. The latter procedure is to be preferred, but the former procedure is often necessary at the beginning. When I speak of precipitating the enzyme I mean, of course, a more or less specific precipitation and not a general

precipitation of everything, such as occurs on adding a great excess of alcohol or acetone.

Some day every enzyme in living matter will have been discovered and described. Every chemical reaction which goes on will have been recorded. We can probably expect to find some enzymes which are glycoproteins, others which are lipoproteins and others which are nucleoproteins.

In 1917 the rôle played by enzymes was only partially understood. Digestive enzymes were well known, autolytic and oxidative enzymes were somewhat known, but not well understood. At present we realize the tremendous complexity of the cell. In muscle alone some sixty enzymes are known to occur. Thanks to relatively recent investigation, practically all of the complicated reactions involved in the breaking down of glycogen to carbon dioxide and water have been made clear.

The organic chemist has never been able to synthesize cane sugar, but by using enzymes, the biological chemist can synthesize not only cane sugar but also gum dextran, gum levan, starch and glycogen.

We know now of the existence of enzymes which employ phosphoric acid instead of water and which might be given the general term «phosphorases». There are the phosphorylases, transphosphorylases, phosphoisomerases, phosphomutases and phosphodismutases.

From the work of Cori and his associates we now have evidence that hormones function through their effect on enzymes. Thus, glucose is transformed into glucose-6-phosphate when it reacts with ATP in the presence of the enzyme known as hexokinase. This reaction, essential for utilization of glucose, is inhibited by the diabetogenic hormone coming from the anterior pituitary. This inhibiting action is abolished by insulin.

We can sum up by saying that as the result of discoveries in the field of enzyme chemistry some questions have been answered and many new questions have arisen. We live in an expanding universe in more senses than that of the astronomers.

In conclusion I wish to pay tribute to my former teacher, Otto Folin, who emigrated as a boy of seventeen from Småland to America and who, as Professor of Biochemistry at Harvard, inspired me as he did many other young men.

Biography

James Batcheller Sumner was born at Canton, Mass., on Nov. 19, 1887, as the son of Charles Sumner and Elizabeth Rand Kelly. His ancestors were Puritans who came from Bicester, England, in 1636 and settled in Boston. His father owned a large country estate, while his grandfather had a farm and also a cotton factory. Young Sumner attended the Eliot Grammar School for a few years and then was sent to Roxbury Latin school. At school he was bored by almost every subject except physics and chemistry. He was interested in fire-arms and often went hunting. While grouse hunting at the age of 17, he was accidentally shot in the left arm by a companion; as a consequence, his arm had to be amputated just below the elbow. Having been left-handed, he then had to learn to do things with his right hand. The loss of his arm made him exert every effort to excel in all sorts of athletic sports, such as tennis, skiing, skating, billiards, and clay-pigeon shooting.

In 1906 Sumner entered Harvard College; he graduated in 1910, having specialized in chemistry. After a short interval of working in the cotton knitting factory owned by his uncle, a type of work that did not interest him in the least, he accepted a teaching post at Mt. Allison College, Sackville, New Brunswick. This was followed by an assistantship in chemistry at Worcester Polytechnic Institute, Worcester, Mass., in 1911, from which he resigned in 1912 in order to study biochemistry with Professor Otto Folin at Harvard Medical School. Although Folin advised him to take up Law, since he thought that a one-armed man could never make a success of chemistry, Sumner persisted and obtained his Ph.D. degree in June, 1914. A few months later while travelling in Europe he was stranded in Switzerland for about a month by the outbreak of World War I. During this time he received a cable inviting him to be Assistant Professor of Biochemistry at Cornell Medical School, Ithaca, N.Y., a post which he held until 1929, when he was made full Professor of Biochemistry.

Sumner's research work at Cornell first centered around analytical methods; despite hard work he was unable to obtain any interesting results. He then decided to isolate an enzyme in pure form, an ambitious aim never

achieved by anyone up till then, but a type of research suited to his scanty apparatus and very small laboratory staff. In particular, he worked with urease.

For many years his work was unsuccessful, but in spite of discouragement from colleagues who doubted whether any enzyme could ever be isolated in pure form he continued. In 1921, when his research was still in its early stages, he had been granted an American-Belgian fellowship and decided to go to Brussels to work with Jean Effront, who had written several books on enzymes. The plan fell through, however, because Effront thought Sumner's idea of isolating urease was ridiculous. Back in Ithaca, he resumed his work until finally, in 1926, he succeeded («I went to the telephone and told my wife that I had crystallized the first enzyme», he wrote in an autobiographical note). His isolation and crystallization of urease met with mixed response; it was ignored or disbelieved by most biochemists, but it brought him a full professorship in 1929.

Gradually, recognition came. In 1937, he was given a Guggenheim Fellowship; he went to Uppsala and worked in the laboratory of Professor The Svedberg for five months. He was awarded the Scheele Medal in Stockholm in the same year. When Northrop, of the Rockefeller Institute, obtained crystalline pepsin, and subsequently other enzymes, it became clear that Sumner had devised a general crystallization method for enzymes. The opponents gradually admitted Sumner's and Northrop's claims – Willstätter last of all – and the crowning recognition came in 1946 when the Nobel Prize was awarded to Sumner and Northrop. In 1948, Sumner was elected to the National Academy of Sciences (USA).

Sumner was married three times: in 1915 he married Bertha Louise Ricketts whom he later divorced. They had six children, one of whom died at an early age. In 1931 he married Agnes Paulina Lundkvist, and finally in 1943 Mary Morrison Beyer. He died of cancer on August 12, 1955.

John H. Northrop

The preparation of pure enzymes and virus proteins*

Nobel Lecture, December 12, 1946

The problem of the chemical nature of the substances which control the reactions occurring in living cells has been a subject of research, and also of controversy, for nearly two hundred years. Before the eighteenth century these reactions were considered as «vital processes», outside the realm of experimental science. The work of Spallanzani, Payen and Persoz, Schwann, Kühne, and finally Buchner proved that many of these reactions could take place without living cells and were probably caused by the presence of small amounts of unstable and active substances, which Kühne called «enzymes».

Berzelius, a century ago, pointed out that these enzymes were similar to the catalysts of the chemist and suggested that they be considered as special catalysts formed by the cells. This hypothesis was far ahead of its time and met with great opposition, since many workers considered that enzyme reactions differed qualitatively from ordinary chemical reactions. The work of Tamman, Arrhenius, Henri, Michaelis, Nelson, von Euler, Willstätter, Warburg, and other chemists, however, has shown that Berzelius' viewpoint was correct and enzyme reactions are now considered a special kind of catalysis which does not differ qualitatively from other catalytic reactions.

While the study of enzyme reactions made rapid progress all attempts to isolate an enzyme and so determine its chemical nature were unsuccessful until recently.

The early workers were of the opinion that enzymes were probably proteins and in 1896 Pekelharing isolated a protein from gastric juice which he considered to be the enzyme pepsin. He was not able to crystallize the protein and his conclusion as to the identity of the enzyme and the protein was never accepted. I repeated these experiments about 1920 but was unable to carry them further at that time. In the meantime a large amount of work,

* A detailed description of the results described in this paper may be found in *Crystalline Enzymes*, by J. H. Northrop, M. Kunitz, and R. M. Herriott, Columbia University Press, New York, 2nd ed., 1948. This book also contains complete references to the literature.

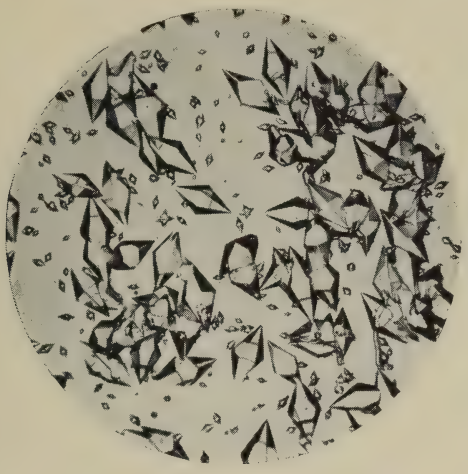


Fig. 1. Crystalline pepsin (× 90). Northrop, 1930.

Fig. 2. Crystalline pepsin from 15 per cent alcohol (× 225). Northrop, 1946.

principally by Willstätter and his students, led to the conclusion that enzymes were a special class of unknown compounds, and certainly not proteins.

However, in 1926 Sumner isolated a crystalline protein from beans which he considered to be the enzyme urease. This conclusion was also received with skepticism but, as you know, it has turned out to be perfectly correct. Professor Sumner, himself, has reported this work to you.

Sumner's results encouraged me to take up the pepsin problem again and in 1930 I isolated a crystalline protein from a commercial pepsin preparation which appeared to be the enzyme pepsin. Since then five more enzymes, as well as some of their precursors, have been isolated in my laboratory. Trypsin, and its precursor trypsinogen, a polypeptide which inhibits trypsin, and a compound of this substance with trypsin, chymotrypsinogen, and three forms of chymotrypsin were isolated and crystallized by Kunitz and myself. Kunitz and McDonald isolated and crystallized ribonuclease and hexokinase. Anson crystallized carboxypeptidase, and Herriott isolated and crystallized pepsinogen. These experiments required a great deal of the most painstaking and difficult work and could not have been successfully carried out without my collaborators, Herriott, Anson, Desreux, McDonald, Holter, Krueger, Butler, and, especially, Dr. Kunitz, who possesses a real genius for handling these unstable and elusive substances.

In the meantime some twenty enzymes have been crystallized in all by other workers; all of these enzymes are proteins. The respiratory ferments of Warburg and other oxidative enzymes contain special groups other than

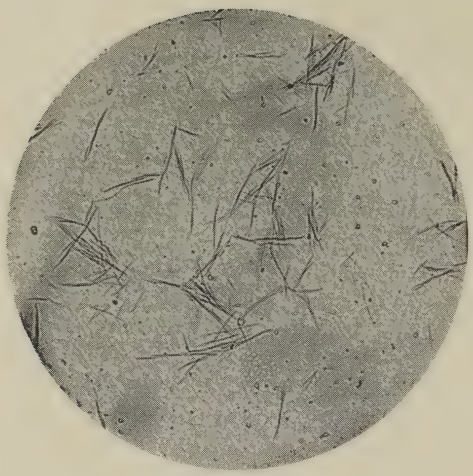


Fig. 3. Crystalline 60 per cent active acetyl pepsin (× 28). Herriott & Northrop, 1933.

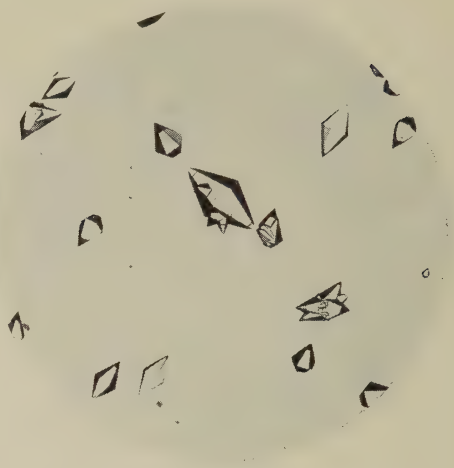


Fig. 4. Crystalline pepsinogen (× 340). Herriott, 1936.

amino acids, but the purely hydrolytic enzymes, so far as is known, do not.

While this work was in progress a new controversy, very similar to the old controversy over the nature of enzymes, arose concerning the chemical nature of viruses. Dr. Stanley succeeded in crystallizing the virus of tobacco mosaic and has isolated a number of other viruses. Bawden and Pirie have crystallized the bushy stunt virus of tomatoes. I have isolated a nucleoprotein which appears to be one of the bacterial viruses, or bacteriophages. This is Dr. Stanley's field and he will report to you concerning it.

As a result of these experiments, it appears probable that all enzymes and at least some viruses are proteins. The mere fact that the preparations are crystalline proteins is not, of course, sufficient to warrant this conclusion and we have spent a great deal of time in establishing the purity of our preparations and in testing by every method available the relation between the activity and the protein.

Before discussing these results, I will describe briefly the experimental methods we have used in the isolation and crystallization of these active proteins. No one general method has been found which will lead to the isolation and crystallization of an enzyme, but certain general principles have been found to be of great assistance. In the first place large quantities of material are used, so that actual solid material is handled and not merely dilute solutions. The failure of so many early attempts to isolate enzymes was due, I believe, largely to the fact that nearly all of the work was carried out with

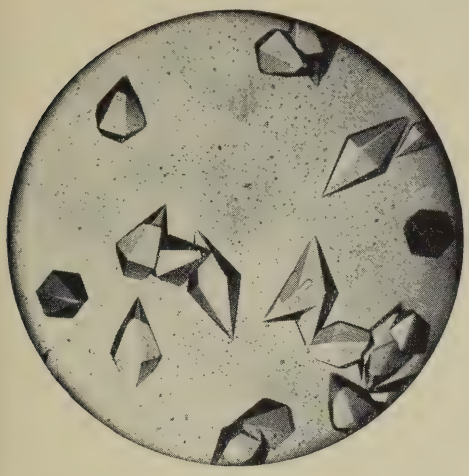


Fig. 5. Crystalline active acetyl pepsin (× 34). Herriott & Northrop, 1934.

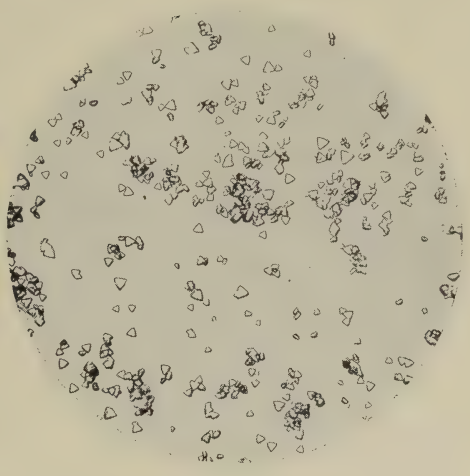


Fig. 6. Crystalline trypsinogen (× 220). Kunitz & Northrop, 1935.

dilute solutions. In the second place filtration by suction was used wherever possible since this method results in far better separation of the precipitate from the mother liquor than does the use of the centrifuge. Had Pekelharing filtered his pepsin preparation and then dissolved it in a very small amount of water, instead of centrifuging the precipitate and dissolving it in a large amount of water, I am quite sure he would have crystallized the enzyme nearly fifty years ago.

In the third place fractionation was carried out largely by the use of concentrated neutral salts in the presence of which proteins are much more stable than in dilute salt solutions.

Pepsin, for instance, decomposes at the rate of about 3 per cent a day in hydrochloric acid solution at pH 2.7 and 0° C. Even in nearly saturated magnesium sulfate solutions at 0° C, the most stable condition known, the rate is 1 per cent a day.

Trypsin at pH 8.0 and 30° C loses 90 per cent or more of its activity in a day. This reaction is exceptional since it is bimolecular so that in this case dilute solutions are more stable than concentrated ones.

Tests of purity

Solubility. There is no doubt that the crystalline materials isolated by Sum-

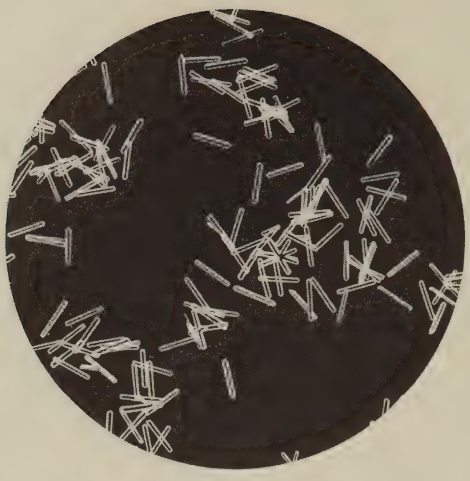


Fig. 7. Crystalline trypsin (× 200). Kunitz & Northrop, 1935.

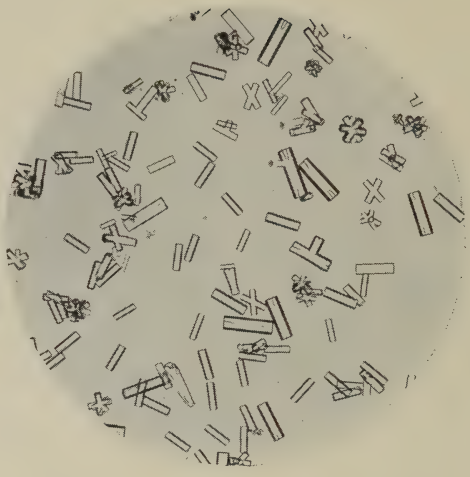


Fig. 8. Crystalline trypsin-inhibitor (× 225). Kunitz & Northrop, 1935.

ner, myself, and others are proteins but the proof that these proteins are the enzymes, themselves, is open to question owing to the difficulty of establishing the purity of a protein. In organic chemistry constant properties, composition, and melting point, through repeated fractional crystallization are considered as sufficient proof of purity. In the case of proteins, constant analysis means very little and the melting point, which is the most sensitive test, cannot be used since proteins decompose instead of melting. However, the solubility of the protein, which, from the point of view of the phase rule, is strictly analogous to the melting point, may be determined instead.

According to Willard Gibbs's phase rule, a system consisting of a single solid in equilibrium with its solution is of fixed composition. That is to say, if more of the same solid is added no change in concentration will occur. If any other substance is added, however, a change will occur. The test is exceedingly specific, even more so than the serological tests as Landsteiner and Heidelberger pointed out. It will, in fact, distinguish between optical isomers, even though the two isomers have exactly the same solubility. In addition, the concentration of the active material as well as the protein may be determined. From the experimental point of view it has the great advantage that the test may be carried out in concentrated salt solution, in which the proteins are most stable. Very few pure proteins have ever been obtained, as judged by this test. Of these, chymotrypsinogen is probably the best. Pepsin, pepsinogen, chymotrypsin, trypsin, ribonuclease, and hexokinase all give

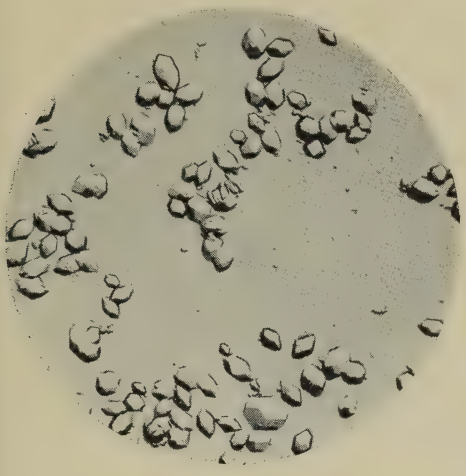


Fig. 9. Crystalline inhibitor and trypsin compound (× 370). Kunitz & Northrop, 1935.

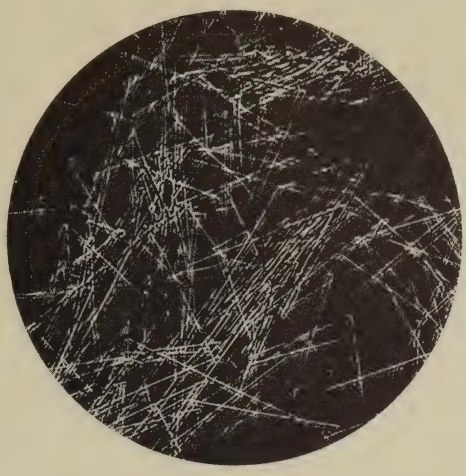


Fig. 10. Crystalline chymotrypsinogen (× 260). Kunitz & Northrop, 1934.

solubility curves closely approaching those of a single substance and as good or better, than those obtained with most other proteins.

The fact that strictly homogeneous proteins are so difficult to prepare indicates that very closely related groups of proteins, and not one particular individual, are synthesized by the organism. The recent interesting results of Pedersen and of Wyman, Rafferty, and Ingalls, who found that the proteins of young animals differ from those of old animals, confirm this explanation. It is very unlikely that an animal synthesizes the «young» protein for a certain time and then suddenly synthesizes the «old» protein and hence it is probable that the various kinds of protein are present at the same time in any one animal.

Analysis in the ultracentrifuge. The many important and striking results which Professor Svedberg has obtained with the ultracentrifuge are familiar to all of you. I need only say that it is a very valuable criterion of homogeneity in protein solution. There is an experimental difficulty in the case of the extremely unstable proteins with which we are involved in that concentrated salt solutions cannot be used owing to their high specific gravity, and some of the proteins are too unstable in dilute salt, even at 0° C, to enable the measurement to be made. Nevertheless, the enzymes pepsin (Philpot and Eriksson-Quensel), ribonuclease and hexokinase (Rothen), and the bacteriophage nucleoprotein (Wyckoff) were found to be homogeneous by this test.

Fig. 11. Crystalline chymotrypsin (× 100). Kunitz & Northrop, 1934.

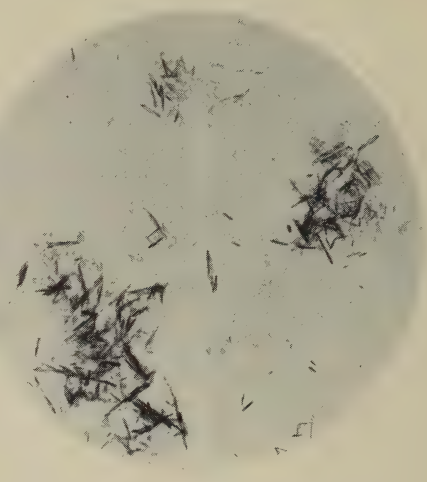


Fig. 12. Crystalline beta chymotrypsin (× 300). Kunitz, 1937.

Electrophoresis. The elegant electrophoresis technique of Tiselius also needs no description in Stockholm. Ribonuclease, hexokinase, and chymotrypsinogen have been examined by Rothen and found to be homogeneous. Tiselius, Henschen, and Svensson studied the electrophoresis of pepsin and found the main protein component to be strictly homogeneous under a wide variety of conditions, but some inactive nitrogenous material was separated from the protein by this method.

Ågren and Hammarsten found that the enzyme migrated to the anode at pH 3.4 and to the cathode at pH 2.7. Tiselius, Henschen, and Svensson, on the other hand, found the protein migrated always to the anode, while I had found by cataphoresis experiments that the protein was isoelectric at about pH 2.4. The electrophoresis experiments were repeated by Herriott, Desreux, Rothen, and Longsworth, using a specially purified pepsin preparation, and both the Tiselius, and the Theorell apparatus. The experiments showed that the various conflicting results were due to decomposition products in the pepsin preparations. In the presence of those products the enzyme has an isoelectric point at pH 2.5 to 3.0, whereas the pure protein has no determinable isoelectric point. This marked effect of hydrolysis products on the electrophoresis of pepsin had been observed many years ago by Ringer. These non-protein decomposition products may be removed partially by electrophoresis but we could find no evidence for the presence of more than one protein by this method.

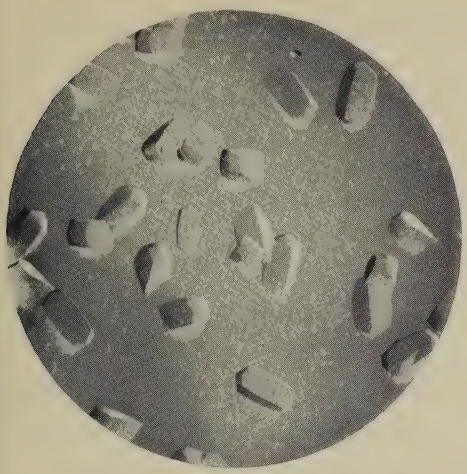


Fig. 13. Crystalline gamma chymotrypsin (× 15). Kunitz, 1937.

Fig. 14. Crystalline carboxypeptidase (× 85). Anson, 1936.

Ågren and Hammarsten were also able to separate crystalline carboxypeptidase into several components, so it appears that this enzyme has not yet been prepared in pure form.

The results of these various tests of purity warrant the conclusion, I believe, that the enzymes pepsin, trypsin, chymotrypsin, hexokinase, and ribonuclease are at least as homogeneous as any known proteins. Chymotrypsinogen is probably the purest protein so far isolated. This statement refers only to specially prepared and highly purified samples. The first crystallization usually results in a product which still contains impurities and in some cases more than one protein.

Relation of the activity to the protein by other methods

Formation of enzymes from their precursors. Pepsin, trypsin, and chymotrypsin are derived from inactive precursors. These precursors were isolated and crystallized and the formation of the active enzyme studied. The formation of pepsin from pepsinogen and trypsin from trypsinogen are autocatalytic reactions. These enzymes may therefore be « propagated », just as are bacteria. The formation of trypsin from trypsinogen may also be catalyzed by enterokinase, an enzyme of the digestive tract, or by an enzyme produced by a mold (*Penicillium.*) The formation of chymotrypsin from chymotrypsinogen

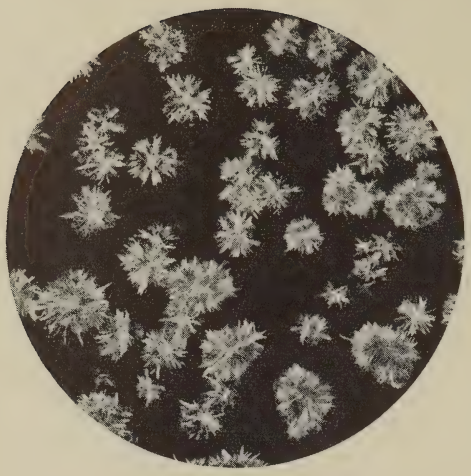


Fig. 15. Crystalline ribonuclease (× 200). Kunitz, 1939.

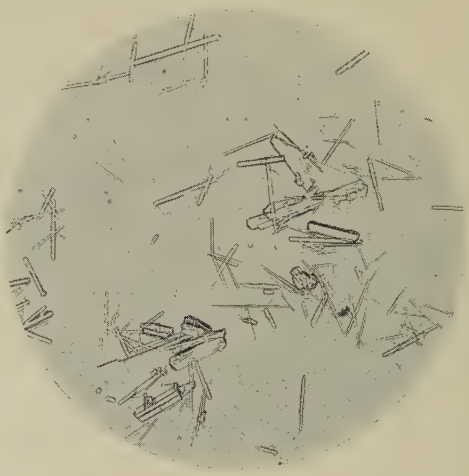


Fig. 16. Crystalline hexokinase (× 100). Kunitz & McDonald, 1942.

is catalyzed only by trypsin, so far as is known. In all these reactions the increase in enzymatic activity is accompanied quantitatively by the appearance of the new enzyme protein which is quite different in all its properties from the original precursor. It seems to me that these results are perhaps the most convincing evidence that the enzymatic activity is actually a property of the protein molecule.

Rate of diffusion of the protein and the active substance. The relationship of the protein and the enzyme may be tested further by measuring the rate of diffusion of the preparation by means of protein determinations and also by enzyme activity measurements. The rate of diffusion was suggested by Arrhenius years ago as a means of determining the size of unknown substances. The method has been developed and used by von Euler.

Theoretically it is simple but experimentally it was difficult. These difficulties were overcome by Anson and myself, who developed a diffusion cell made of a porous disk across which diffusion takes place. This greatly accelerates and simplifies the experiment. The method is the only one, I believe, which permits the estimation of the molecular weight of unknown and impure substances.

The diffusion of pepsin, trypsin, chymotrypsin, chymotrypsinogen, and bacteriophage has been followed by this method. The results show that the protein and the active substance diffuse at exactly the same rate.

Effect of hydrolysis of the protein on the enzymatic activity. Digestion of tryp-

sin or ribonuclease with pepsin results in loss of activity which is almost exactly parallel to the loss of protein. There is no indication that the protein may be hydrolyzed without also destroying the enzymatić activity, nor that any of the hydrolysis products possess measurable activity. Similar results were obtained during the autolysis of pepsin and the digestion of bacteriophage with chymotrypsin.

Denaturation of the protein and loss of activity. The denaturation of proteins is a characteristic property and has an extremely high temperature coefficient. The rate of formation of denatured protein and the loss in enzymatic activity has been compared in the case of pepsin, trypsin, chymotrypsin, ribonuclease and hexokinase. In every case the loss of native protein is accompanied by a concomitant loss of enzymatic activity.

An equilibrium exists between native and denatured trypsin which is accurately predicted by Van't Hoff's equation.

Reversal of the reaction results in recovery of the enzymatic activity in proportion to the recovery of native protein.

Effect of chemical changes in the protein molecule on the activity. Ketene reacts with pepsin to form a series of acetylated products. Herriott has isolated and crystallized three of these derivatives. One contains three or four acetyl groups, probably attached to the primary amino groups. This derivative has the same activity as the original pepsin. A second derivative has about ten acetyl groups and 60 per cent of the original activity, while the third preparation contains twenty to thirty acetyl groups and is inactive. There is evidence that the acetyl groups which are attached to the hydroxyl group of tyrosine are responsible for the loss of activity. This conclusion is borne out by the fact (Herriott) that iodination of pepsin results in the addition of iodine to the tyrosine groups, and also results in loss of activity.

Pepsin, as well as other enzymes, is also inactivated by mustard gas (dichlor ethyl sulfide). This substance is an excellent protein reagent. It reacts in slightly acid solution with carboxyl groups and probably with the hydroxyl group of tyrosine.

Summary

The enzymes, pepsin, trypsin, chymotrypsin, carboxypeptidase, ribonuclease, and hexokinase have been isolated and crystallized. The precursors of pepsin, trypsin, and chymotrypsin have also been isolated and crystallized. A

nucleoprotein which appears to be bacteriophage has been isolated but not crystallized.

The purity of the enzyme preparations, with the exception of carboxypeptidase, has been tested by means of solubility measurements, ultracentrifuge analysis (pepsin, ribonuclease, hexokinase, and bacteriophage), and electrophoresis (pepsin, ribonuclease, hexokinase, chymotrypsinogen). They appear to be pure proteins by all these methods.

The relation of the enzymatic activity to the protein has been tested by diffusion measurements (pepsin, trypsin, chymotrypsin, chymotrypsinogen, bacteriophage), denaturation of the protein (pepsin, trypsin, chymotrypsin, ribonuclease, hexokinase), hydrolysis of the protein (trypsin, ribonuclease, pepsin, bacteriophage), formation of the active enzyme from an inactive precursor (pepsin, trypsin, chymotrypsin), and by the formation and isolation of definite chemical derivatives of pepsin. These experiments confirm the conclusion that the enzymatic activity is a property of the protein molecule itself, and is not due to a non-protein impurity.

Biography

John Howard Northrop was born in Yonkers, New York, on July 5th, 1891. He is a direct descendant of Joseph Northrop who settled in New Milford, Connecticut in 1639, of Jonathan Edwards, President of Princeton University, 1758, and of Frederick C. Havemeyer, whose family presented Havemeyer Hall, the Chemical Laboratory, to Columbia University. His father, John I. Northrop, an instructor at Columbia, was fatally injured in a laboratory accident shortly before his birth. His mother, Alice R. Northrop, who formerly taught botany at Hunter College, New York City, returned to teaching and was responsible for the introduction of nature study into the curriculum of New York public schools. Her former country home in Massachusetts is maintained by the « Northrop Memorial » as a school of nature study for New York schoolchildren.

After public school education, Northrop entered Columbia University in 1908 to study zoology and chemistry under T. H. Morgan and J. M. Nelson. He graduated Bachelor of Science in 1912, Master of Arts in 1913 and received his Ph. D. in chemistry in 1915. He was appointed W. B. Cutting Travelling Fellow and spent the next year in Jacques Loeb's laboratory at the Rockefeller Institute. The following year, he was appointed to the staff of the Institute and with the exception of his war service as a Captain in the Chemical Warfare Service (1917–1918), he has remained with the Institute ever since, becoming an Associate in 1917, Associate Member in 1920, and Member in 1924. In 1949 he was appointed Professor of Bacteriology, University of California, and later, Professor of Biophysics.

Northrop's researches at Columbia were chiefly concerned with carbohydrates and his early work at the Rockefeller Institute was connected with theories of duration of life. Whilst in the service, he discovered a fermentation process for acetone, which he developed to the pilot-plant stage, and on demobilization and return to the Institute, he worked with Loeb on a kinetical study of enzymes essential to life processes. In 1929 he isolated pepsin in pure crystalline form by techniques which were later used by himself and other workers to crystallize trypsin, chymotrypsin, carboxypeptidase, and

pepsinogen. He studied the proteins of viruses and antibodies, and succeeded in isolating a nucleoprotein which had bacteriophage activity: his suggestion that the activity was due to nucleic acid was later proved to be correct. During World War II, he was consultant to the National Defense Research Committee and he studied the mode of action of war gases and methods of detection, developing an apparatus for their automatic detection and analysis. His more recent researches have included work on the origin and relationship of viruses and the transforming principle.

Professor Northrop is the author of *Crystalline Enzymes*, published in 1939. He edited the *Journal of General Physiology* (Rockefeller Institute) for some years and he has written numerous papers on the physical chemistry of proteins, agglutination of bacteria, kinetics of enzyme reaction, and the chemical nature of enzymes.

He was awarded the Stevens Prize (Columbia) in 1931; Chandler Medal, 1936; Daniel Giraud Elliot Medal, 1939; the Certificate of Merit (U.S. Government), 1948; Alex. Hamilton Medal, 1961. He has received honorary Doctor of Science degrees from the Universities of Harvard, Columbia, Yale, Princeton, and Rutgers; and honorary Doctor of Law from the University of California. He is Honorary Fellow of the Chemical Society (London) and a member of many other scientific societies.

Professor Northrop married Louise Walker in 1917. Their only son, John, is an oceanographer and their only daughter, Alice, married Professor Frederick C. Robbins, Nobel Laureate in Medicine, 1954. He is fond of riding and sailing, plays golf and tennis, but his chief hobbies are field shooting and salmon fishing.

Wendell M. Stanley

The isolation and properties of crystalline tobacco mosaic virus

Nobel Lecture, December 12, 1946

Although the idea that certain infectious diseases might be caused by invisible living agents was expressed by Varro and Columella about 100 B. C., there was no experimental proof and the idea was not accepted. The cause of infectious disease remained a mystery for hundreds of years. Even the wonderful work of Leeuwenhoek and his description of small animals and bacteria during the years from 1676 to 1683 failed to result in proof of the relationship between bacteria and infectious disease. There was, of course, much speculation and during the latter half of the 19th century great controversies arose over the germ theory of disease. Then, through the brilliant work of Pasteur, Koch, Cohn, Davaine and others, it was proved experimentally, for the first time, that microorganisms caused infectious diseases. The Golden Era of bacteriology followed and the germ theory of infectious disease was accepted so completely that it became heresy to hold that such diseases might be caused in any other way. Thus, when, in 1892 Iwanowski discovered that the juice of a plant diseased with tobacco mosaic remained infectious after being passed through a filter which retained all known living organisms, he was willing to conclude that the disease was bacterial in nature, despite the fact that he could not find the causative bacteria. As a result, his observations failed to attract attention. However, six years later, the filtration experiment was repeated and extended, independently, by Beijerinck, who immediately recognized the significance of the results and referred to the infectious agent, not as being bacterial in nature, but as a *contagium vivum fluidum*. Obviously, Beijerinck was quite aware of the fact that he had discovered a new type of infectious disease-producing agent. It is also obvious, from the terminology that he used, that he thought of this agent as a living entity.

Since the original discovery of this infectious, disease-producing agent, known as tobacco mosaic virus, well over three hundred different viruses capable of causing disease in man, animals and plants have been discovered. Among the virus-induced diseases of man are smallpox, yellow fever, dengue fever, poliomyelitis, certain types of encephalitis, measles, mumps, influenza,

virus pneumonia and the common cold. Virus diseases of animals include hog cholera, cattle plague, foot-and-mouth disease of cattle, swamp fever of horses, equine encephalitis, rabies, fowl pox, Newcastle disease of chickens, fowl paralysis, and certain benign as well as malignant tumors of rabbits and mice. Plant virus diseases include tobacco mosaic, peach yellows, aster yellows, potato yellow dwarf, alfalfa mosaic, curly top of sugar beets, tomato spotted wilt, tomato bushy stunt, corn mosaic, cucumber mosaic, and sugar cane yellow stripe. Bacteriophages, which are agents capable of causing the lysis of bacteria, are now regarded as viruses.

The viruses have been separated as a special group of infectious, disease-producing agents by means of several general properties, no one of which is, however, exclusively characteristic of viruses. Nevertheless, no great amount of difficulty has been encountered in the segregation of the virus group. Viruses are characterized by their small size, by their ability to reproduce or multiply when within the living cells of a given host, by their ability of change or mutate during multiplication and by their inability to reproduce or grow on artificial media or in the absence of specific living cells. The sole means of recognizing the existence of a virus is provided by the multiplication of the virus which is, of course, usually accompanied by manifestations of disease. Viruses spread from diseased to normal susceptible hosts by different methods. Some are transferred by direct contact, as when a diseased leaf is caused to rub against a healthy leaf by a gust of wind, or when a normal person or animal comes into direct contact with a diseased person or animal. Such viruses can usually be spread by indirect contact through the medium of non-specific animate or inanimate objects. Some viruses cannot be transferred by direct contact, but require an intermediate host such as a mosquito, louse or leaf-hopper. In some cases a highly specific intermediate host is necessary, and a more or less definite period of incubation within this host may be required before it can pass on the virus.

Reproduction, mutation and metabolic activity have long been regarded as unique and special properties of living organisms. When viruses were found to possess the ability to reproduce and to mutate, there was a definite tendency to regard them as very small living organisms, despite the fact that the question of metabolic activity remained unanswered. Because of their small size they could not be seen by means of the ordinary light microscope. Although this fact puzzled some investigators, it was pushed aside and for over thirty years interest in virus research was centered about the discovery of new viruses and on studies of the pathological manifestations of viruses.

Approximate sizes of viruses and reference materials.

	Diameter or width × length in mμ
Red blood cells	*7500*
B. prodigiosus (Serratia marcescens)	*750*
Rickettsia	475
Psittacosis	450
Canary pox	260 × 310
Myxoma	230 × 290
Vaccinia	210 × 260
Pleuro-pneumonia organism	*150*
Pseudo rabies	150
Herpes simplex	150
Rabies fixe	125
Influenza	115
Newcastle disease	115
Vesicular stomatitis	100
Staphylococcus bacteriophage	100
Fowl Plague	90
T_2 coli bacteriophage	60 × 80
Chicken tumor I	70
Equine encephalomyelitis	50
T_3 coli bacteriophage	45
Rabbit papilloma (Shope)	44
Pneumonia virus of mice	40
Tobacco mosaic and strains	15 × 280
Latent mosaic of potato	10 × 525
Gene (Muller's est. of max. size)	*20 × 125*
Southern bean mosaic	31
Rift valley fever	30
Tomato bushy stunt	26
Poliomyelitis (Lansing)	25
Hemocyanin molecule (Busycon)	*22*
Yellow fever	22
Hemocyanin molecule (Octopus)	*20*
Louping ill	19
Tobacco ring spot	19
Japanese B encephalitis	18
Alfalfa mosaic	17
Tobacco necrosis	16
Foot-and-mouth disease	10
Silkworm jaundice	10
Hemoglobin molecule (Horse)	*3 × 15*
Egg albumin molecule	*2.5 × 10*

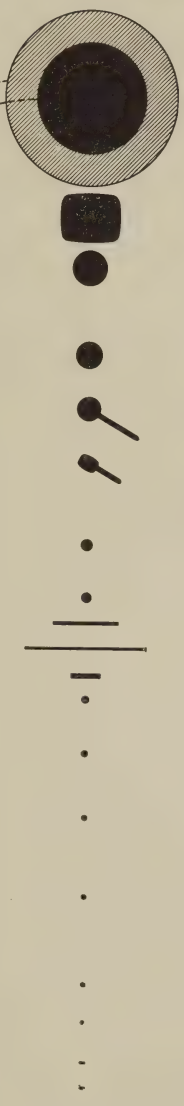


Fig. 1. A chart showing approximate sizes of several viruses and reference materials. (From W. M. Stanley, *Chem. Eng. News*, 25 (1947) 3786.)

Then, around 1930, Elford began his important work on the filtration of viruses through graded collodion membranes. He demonstrated that different viruses possessed different and characteristic sizes, and that some viruses were as large as about 300 mμ, whereas others were as small as 10 mμ. It was soon realized that the acceptance of a virus 10 mμ in size as a living organism presented certain inherent difficulties, especially with respect to metabolic activity. Grave doubts were expressed that the complicated processes of respiration and digestion and the general metabolic functions usually associated with life could be contained within structures as small as 10 mμ, especially since protein molecules larger than 10 mμ were known. It can be seen from Fig. 1, which is a chart illustrating the relative sizes of several viruses and certain reference materials, that the viruses overlap with respect to size, not only with protein molecules, but also, at the other extreme, with accepted living organisms. For example, several viruses are smaller than certain hemocyanin protein molecules, and several viruses are larger than the pleuropneumonia organism, which is an accepted living organism capable of growth on artificial media. The fact that, with respect to size, the viruses overlapped with the organisms of the biologist at one extreme and with the molecules of the chemist at the other extreme only served to heighten the mystery regarding the nature of viruses. Then too, it became obvious that a sharp line dividing living from non-living things could not be drawn and this fact served to add fuel for discussion of the age-old question of « What is life? ».

Attempts to learn something about the nature of viruses through studies on their general properties began with Beijerinck's work in 1898 and were continued in different laboratories for over thirty years without too much success. Although Beijerinck and Allard made important contributions, perhaps the most significant work was that of Vinson and Petre during the years from 1927 to 1931 when they showed that tobacco mosaic virus could be subjected to several kinds of chemical manipulations without loss of virus activity. Nevertheless, when the work on viruses, which is recognized by the 1946 Nobel Prize for Chemistry, was started in 1932, the true nature of viruses was a complete mystery. It was not known whether they were inorganic, carbohydrate, hydrocarbon, lipid, protein or organismal in nature. It became necessary, therefore, to conduct experiments which would yield information of a definite nature. Tobacco mosaic virus was selected for these initial experiments because it appeared to provide several unusual advantages. Large amounts of highly infectious starting material were readily

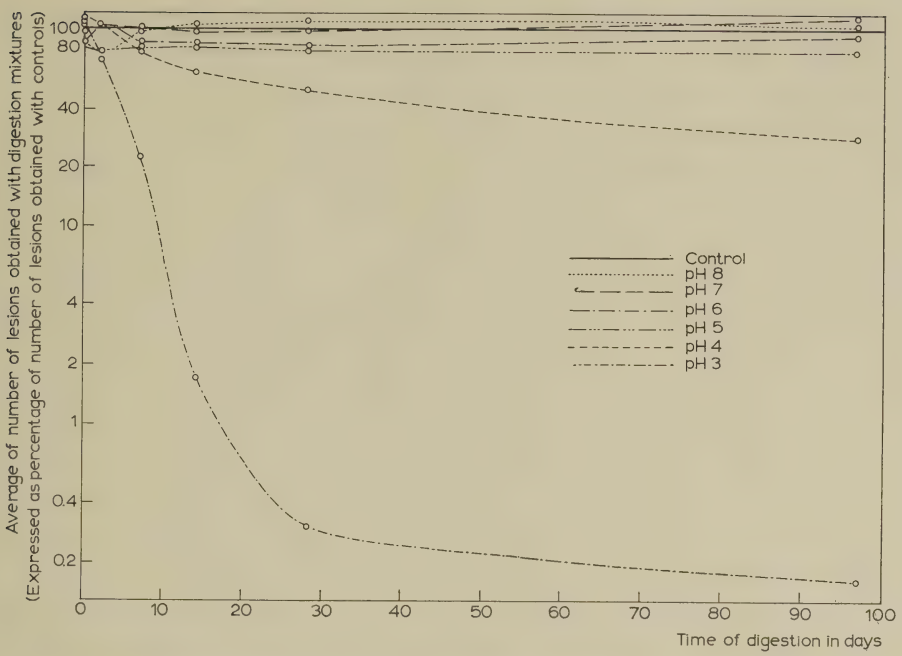


Fig. 2. Graph showing the effect of different hydrogen-ion concentrations on the inactivation of tobacco mosaic virus with pepsin at 37° C. The lines showing the average results obtained at pH 5, 6, 7 and 8 fall very close to the line for the controls, thus indicating little or no inactivation of virus. The line showing the results obtained at pH 3 drops sharply, thus indicating a rapid inactivation of virus. (From W. M. Stanley, *Phytopathology*, 24 (1934) 1269.)

available and the virus was known to be unusually stable. Furthermore, it was possible to titrate or measure the amount of this virus in a preparation with ease and rapidity and with great accuracy. The experimental foundation which resulted in the subsequent isolation of crystalline tobacco mosaic virus was laid during the course of two and one-half years. The work was reported in a series of five papers published in *Phytopathology* in 1934 and 1935 under the general title « Chemical studies on the virus of tobacco mosaic ». The subtitles serve to denote the character of the experimental work and are as follows: I. Some effects of trypsin; II. The proteolytic action of pepsin; III. Rates of inactivation at different hydrogen ion concentrations; IV. Some effects of different chemical agents on infectivity; V. Determination of optimum hydrogen-ion concentrations for purification by precipitation with lead acetate.

The experimental results described in the first paper demonstrate that the

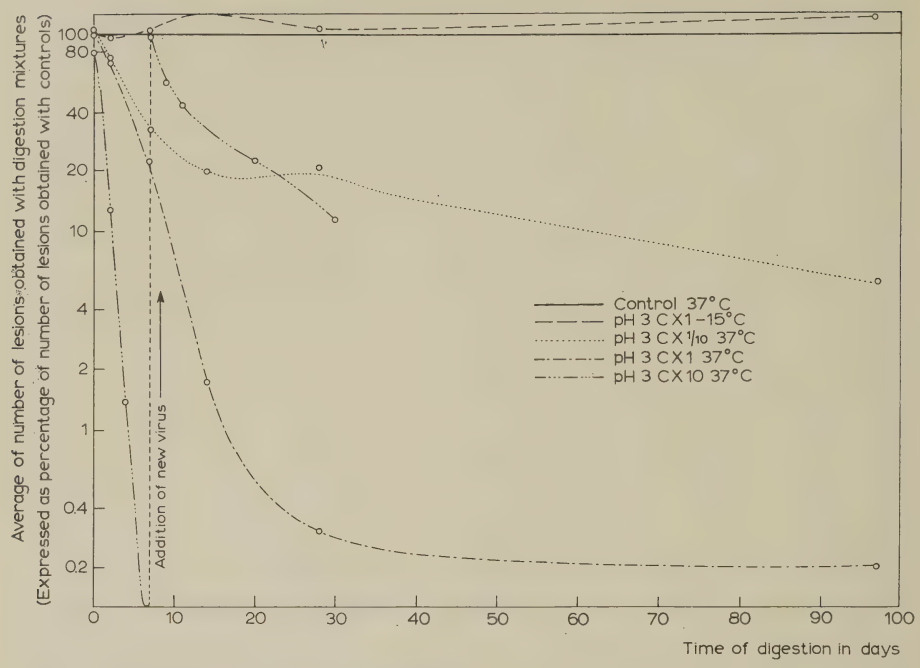


Fig. 3. Graph showing the effect of different pepsin concentrations and of a different digestion temperature on the inactivation of tobacco mosaic virus with pepsin. The line showing the average results obtained with the pepsin concentration ($C \times 1$) used in most of the experiments at 37° C, drops away from the line for the controls sharply, thus indicating a rapid inactivation of virus. The line for the results obtained when the pepsin concentration is reduced to 1/10 ($C \times 1/10$) falls off very gradually, whereas the line for the results obtained when the pepsin concentration is increased 10 times ($C \times 10$) falls off almost at right angles. When new or active virus was added to the latter solutions ($C \times 10$) there was but little immediate effect on the infectivity of the added active virus. However on digestion the activity of the added virus decreased as indicated by the extension of the line for the results obtained with pepsin concentration $C \times 10$. The line for the results obtained with the usual pepsin concentration ($C \times 1$) at −15° C falls very close to the line for the controls, thus indicating little or no inactivation of virus. (From W. M. Stanley, *Phytopathology*, 24 (1934) 1269.)

decrease in infectivity of tobacco mosaic virus on addition of trypsin is not due to the proteolytic activity of trypsin because the loss in infectivity is immediate and can take place over a wide range of hydrogen-ion concentrations, including some at which trypsin is inactive proteolytically. The infectivity of the virus was regained by treatment with heat, by dilution or by digestion and removal of trypsin. It was also found that a similar loss of the infectivity takes place on addition of trypsinogen or globin, proteins which

possess no proteolytic activity, but which, like trypsin, have isoelectric points more alkaline than pH 7. Unfortunately the work with trypsin failed to provide definite information regarding the nature of tobacco mosaic virus. However, in studies with pepsin it was found that this enzyme inactivated the virus only under conditions under which pepsin is active as a proteolytic agent. Pepsin was found to have no appreciable immediate effect on the infectivity of the virus and to have no effect on the infectivity of virus preparations held at pH 7 or pH 8. However, at pH 3 and 37° C the virus activity was found to disappear slowly. Although the rate of inactivation was found to be slower than the rate of peptic digestion of ordinary proteins, it was, nevertheless, proportional to the concentration and activity of pepsin and to the time of digestion. The results of some typical experiments are presented in Figs. 2 and 3. It was not found possible to regain the infectivity of virus inactivated by pepsin. It was concluded in 1934 that «the virus of tobacco mosaic is a protein, or very closely associated with a protein, which may be hydrolyzed by pepsin». At last a definite clue had been obtained and this served to eliminate several kinds of materials from consideration and permitted concentration on proteinaceous materials.

Studies on the rates of inactivation of tobacco mosaic virus at different hydrogen-ion concentrations provided valuable information, for it was found that the virus was extremely stable between pH 3 and pH 8, and fairly stable between pH 1.5 and pH 2.5 and between pH 8 and pH 9. As a result of studies on the effects of over one hundred different chemicals on tobacco mosaic virus, it was found that the only chemicals which had a direct inactivating action could be classified as oxidizing agents, protein-precipitating agents and agents causing a hydrogen-ion concentration known to inactivate the virus. It was concluded that «as a whole, the results are in harmony with the conception that this virus is a protein». As a result of these studies efforts were directed more definitely towards the development of procedures useful in the concentration and purification of proteins. The optimum hydrogen-ion concentrations for carrying out the three principal steps in the lead acetate process for the purification of tobacco mosaic virus proposed by Vinson and Petre were determined. It was found that the optimum hydrogen-ion concentrations for the lead subacetate precipitation, for the neutral lead acetate precipitation and for the elution of the virus from the neutral lead acetate precipitate, were about pH 9, pH 5.5 and pH 7, respectively. Utilization of this process yielded colorless, partially purified solutions having a virus content equal to, or somewhat greater than that of the starting

material. Subsequently, it was found that concentration and purification of the virus could be effected readily by means of a combination of isoelectric precipitation and salting out with ammonium sulfate. A description of this procedure, which yielded a crystalline material as the end-product, is contained in the following paragraph.

Turkish tobacco plants grown in pots or flats in a greenhouse are inoculated when about 3 or 4 inches high by rubbing one or two leaves with a bandage gauze pad moistened with a virus preparation. About 2 or 3 weeks later, and preferably not more than 4 weeks later, the plants are cut and frozen. The frozen plants are put through a meat grinder, the pulp is allowed to thaw, and the juice pressed out. This and all subsequent steps are carried out in a room held at about 4° C, or if this is impossible, the juice and all containers and materials with which it comes in contact are kept as close to 4° C as possible. The juice is adjusted to pH 7.2 ± 0.2 by adding 0.1 to 1 *N* NaOH, recombined with the press cake, well mixed, and again pressed out. The juice will now be at about pH 6.7 ± 0.2. However, if it be desired, sufficient concentrated disodium phosphate to cause the expressed juice to be at pH 6.7 ± 0.2, may be added directly to the pulp. The extract is filtered through a layer of « Standard » celite about ½ inch thick on a Büchner funnel. The celite filter cake is scraped with the flattened end of a spatula from time to time in order to speed the filtration. The filtrate will be found to contain from about 1 to 2 mg total nitrogen per ml, of which about 0.6 to 1.2 mg per ml is protein nitrogen. The globulin fraction is precipitated by the addition of 30 per cent by weight of ammonium sulfate and removed by filtration with filter paper or more rapidly by means of a thin layer of celite on a Büchner funnel. The precipitate is dissolved in 0.1 *M* phosphate buffer at pH 7 or in sufficient water to give about a 1 per cent solution of protein, and then adjusted to pH 7, and again precipitated with ammonium sulfate. It will be found that considerably less ammonium sulfate will be required to precipitate all virus activity. The amount varies somewhat but approaches 11 per cent by weight. The use of smaller amounts of ammonium sulfate permits inactive protein and pigment to be lost in the filtrate. If celite and a Büchner funnel are used, the celite may be removed before precipitation of the protein by filtration at about pH 7 in the presence of less than 11 per cent ammonium sulfate. After two or three precipitations with ammonium sulfate, or when the filtrate from the ammonium sulfate precipitation becomes practically colorless, the precipitate will usually be found to be only slightly colored. If the precipitate has considerable color after several precipitations with salt, much of the color can be

precipitated and removed by treatment of a solution of the precipitate with minimal amounts of lead subacetate. Following an additional precipitation with the salt, the precipitate is dissolved in water and the solution adjusted to about pH 4. This causes precipitation of the protein, which is removed by filtration through a thin layer of celite. The filtrate contains some pigment and inactive, presumably normal, protein. The celite filter cake containing the protein is suspended in water and adjusted to pH 7 and the celite removed by filtration on a Büchner funnel. The filtrate will be opalescent, practically colorless, and contain about 80 per cent of the virus in the starting material.

Fig. 4. Crystals of tobacco mosaic virus (× 675). (From W. M. Stanley, *Am. J. Botany*, 24 (1937) 59.)

The protein in the filtrate is crystallized by adding slowly, with stirring, sufficient of a saturated solution of ammonium sulfate to cause a slight cloudiness, followed by a solution of 10 per cent glacial acetic acid in one-half saturated ammonium sulfate sufficient to increase the hydrogen-ion concentration to about pH 5. The suspension of the crystals has a very characteristic appearance. When stirred it has a satin-like sheen. The crystals, which are shown in Fig. 4, are very small and may best be observed by means of a microscope at a magnification of about 400 times. Within a few months following the publication of a description of the isolation procedure, the isolation of the crystalline material was confirmed in several laboratories in different parts of the world.

Following the isolation of the crystalline material the immediate and important tasks consisted of proving that virus activity either was, or was not, a specific property of the crystalline material and, in the event of a positive

correlation, of securing a complete chemical, biological and physical characterization of the crystalline material. Much effort has been and continues to be devoted to these two tasks. Needless to say, for a time there was great skepticism that the crystalline material could be tobacco mosaic, due chiefly to the old idea that viruses were living organisms. A wide variety of experimental approaches has been used to test the proposal that the crystalline material represented tobacco mosaic virus. It was found that essentially all of the virus activity present in infectious juice could be isolated in the form of the crystalline material. The virus activity of this material was about 500 times that of the starting material. One ml of a solution containing but 10^{-9} grams of the material has usually proven infectious and occasionally, under favorable conditions, one ml containing only 10^{-14} grams has caused infection. The same crystalline material has been obtained repeatedly from different batches of diseased plants obtained at different times of the year and under different growing conditions. The same material has been obtained from different kinds of plants, such as mosaic-diseased tomato, petunia, spinach and phlox plants. The material has been recrystallized with little or no change in its properties. Fractional crystallization yielded no evidence for inhomogeneity. Fractionation by means of filtration through fine membranes, by means of centrifugation and by means of electrophoresis likewise yielded no evidence for the presence of extraneous material. The crystalline material and virus activity were always directly related on movement in a high-speed centrifuge or in the Tiselius electrophoresis apparatus under a wide variety of conditions. Antiserum to the material was found to give a specific precipitin test and to neutralize virus activity specifically. The virus material was found to bear no serological relationship to material present in extracts of normal tobacco, tomato or phlox plants. When solutions of the material were made more alkaline than about pH 11, or more acid than about pH 2, or were heated to about 75° C the protein was denatured and the virus activity was lost. In these cases, as when tobacco mosaic virus was subjected to strong solutions of urea or of detergents, the rate of denaturation of the protein and the loss of virus activity paralleled each other. The ultraviolet light absorption spectrum of the material was found to coincide with the destruction spectrum of virus activity. Treatment of virus solutions with ultraviolet light, hydrogen peroxide or nitrous acid resulted in loss of activity without accompanying gross chemical and physical changes. The high molecular weight and ability to crystallize were retained. Of special significance was the finding that by treatment with formaldehyde, it was possible to cause measurable

Table 1. Composition of tobacco mosaic virus* and some of its strains**

Amino acid	*Strain of virus*								
	TMV	*M*	*J14D1*	*GA*	*YA*	*HR*	*CV3*	*CV4*	*M.D.***
Alanine	5.1	5.2	4.8	5.1	5.1	6.4		6.1	0.2
Arginine	9.8	9.9	10.0	11.1	11.2	9.9	9.3	9.3	0.2
Aspartic acid	13.5	13.5	13.4	13.7	13.8	12.6		13.1	0.2
Cysteine	0.69	0.67	0.64	0.60	0.60	0.70	0	0	
Cystine	0		0		0	0		0	
Glutamic acid	11.3	11.5	10.4	11.5	11.3	15.5	6.4	6.5	0.2
Glycine	1.9	1.7	1.9	1.9	1.8	1.3	1.2	1.5	0.1
Histidine	0	0	0	0	0	0.72	0	0	0.01
Isoleucine	6.6	6.7	6.6	5.7	5.7	5.9	5.4	4.6	0.2
Leucine	9.3	9.3	9.4	9.2	9.4	9.0	9.3	9.4	0.2
Lysine	1.47	1.49	1.95	1.45	1.47	1.51	2.55	2.43	0.04
Methionine	0	0	0	0	0	2.2	0	0	0.1
Phenylalanine	8.4	8.4	8.4	8.3	8.4	5.4	9.9	9.8	0.2
Proline	5.8	5.9	5.5	5.8	5.7	5.5		5.7	0.2
Serine	7.2	7.0	6.8	7.0	7.1	5.7	9.3	9.4	0.3
Threonine	9.9	10.1	10.0	10.4	10.1	8.2	6.9	7.0	0.1
Tryptophan	2.1	2.2	2.2	2.1	2.1	1.4	0.5	0.5	0.1
Tyrosine	3.8	3.8	3.9	3.7	3.7	6.8	3.8	3.7	0.1
Valine	9.2	9.0	8.9	8.8	9.1	6.2	8.8	8.9	0.2

* Tobacco mosaic virus has been found to contain 1.5 per cent of amide nitrogen and 6 per cent of ribonucleic acid.

** The values given in the table represent percentages of the indicated amino acids. In order to facilitate comparison, the values which are considered to differ significantly from those of TMV are underlined.

*** Mean deviation of the values of single determinations from the averages given. Three to five preparations of each strain were analyzed for each amino acid, with the exception of cysteine, and the results were averaged to give the figures presented. (From C. A. Knight, *J. Biol. Chem.*, 171 (1947) 297.

structural changes in the material and loss of virus activity, but by reversing the structural changes the virus activity was regained. As a whole, the results indicated that the crystalline material was, in fact, tobacco mosaic virus.

Attention was therefore directed to the characterization of the crystalline material. The material was originally reported to be a protein and to contain

about 52 per cent carbon, about 7 per cent hydrogen and about 16 per cent nitrogen, and was later found to contain, in addition, about 0.6 per cent phosphorus and 0.2 per cent sulfur. The fact that nucleic acid could be isolated from the crystalline material was reported by Pirie and coworkers in December, 1936, and by the writer a few days later. Although Pirie and coworkers concluded in 1937 that the crystalline virus material was a nucleoprotein, the presence of nucleic acid as a component of the virus was questioned at first by the writer. However, within a few months the writer became convinced that the nucleic acid could not be removed without causing loss of virus activity and there was general agreement that the virus was a nucleoprotein. Later, Loring, working in the writer's laboratory, demonstrated that about 90 per cent of the phosphorus in tobacco mosaic virus could be isolated in the form of pure pentosenucleic acid. This work, as well as the earlier and also subsequent work, has provided ample justification for regarding tobacco mosaic virus as a nucleoprotein. Elementary chemical analyses of the nucleoprotein are not very significant because most proteins have a similar elementary composition. However, the amino acid composition can be used to characterize a protein material somewhat more definitely. Considerable effort has been devoted to a study of the amino acid composition of tobacco mosaic virus and all or most of the virus has been accounted for. The most recent results are given in the first column of Table 1. It can be noted that the virus does not contain histidine or methionine and that there is no special concentration of basic amino acids.

Early experiments involving osmotic pressure indicated that the purified virus material possessed an unusually large particle size. Subsequently a sample of the purified nucleoprotein was sent to Professor Svedberg who, with Eriksson-Quensel, found the nucleoprotein to have a molecular weight, based on a dissymmetry constant of 1.3, of about 17 millions. Later, Lauffer, in the writer's laboratory, conducted extensive studies on the physicochemical and optical properties of solutions of the virus nucleoprotein. Early determinations of viscosity and sedimentation yielded data which permitted the conclusion that the particles of tobacco mosaic virus consisted of rods, about 12 mμ in diameter and about 400 mμ in length, with a molecular weight of about 40 millions. These results were received with some skepticism because they were based on physicochemical theory, certain aspects of which were relatively untested. However, when the electron microscope became available, pictures of purified tobacco mosaic virus were made, first in Germany and later in the writer's laboratory, and these provided direct evidence of the

existence of the rods 15 by 280 mμ in size. A recent study by means of the electron microscope of tobacco mosaic virus from three different parts of the world showed that the most common length of the rods is 280 ± 8.6 mμ. Electron micrographs of these three samples are shown in Fig. 5. Recent studies on the physicochemical and optical properties of solutions of tobacco mosaic virus have yielded data on the size and shape of the virus rods which are in complete accord with the results obtained with the electron microscope. Because of the high molecular weight of tobacco mosaic virus and because adequate high-speed centrifuges have become available during the past few years, a physical method involving differential centrifugation has been developed for the concentration and purification of tobacco mosaic virus and this method is now used extensively.

An interesting and potentially very important property of the rods of tobacco mosaic virus is their ability to aggregate end-to-end. This aggregation appears to be partly reversible and partly irreversible and the degree of reversibility appears to depend in part upon the conditions under which the particles were originally aggregated. Although electron micrographs of the contents of hair cells of tobacco mosaic diseased plants indicate that about two-thirds of the rod-like particles present are about 280 mμ in length, it is possible to secure purified preparations of tobacco mosaic virus in different stages of aggregation. The degree of aggregation depends largely upon the method of purification and preparations having the highest specific virus activity always consist of monomers, that is, of rods about 280 mμ in length. If these are aggregated, the specific activity or activity per mg of protein nitrogen, is decreased. Likewise, preparations obtained from infectious juice by a process designed to yield a preponderance of rods shorter than 280 mμ, or preparations of short rods obtained by sonic treatment of rods 280 mμ in length, always possess a low or negligible virus activity. Although it is impossible to foretell what the future may bring, the experimental data available at present indicate that the rods 15 by 280 mμ in size represent tobacco mosaic virus, despite the fact that preparations of these rods in different stages of aggregation can exist. Studies on the interaction of the virus rods are proving a fruitful field and may yield significant information regarding important processes within living cells. Bernal and Fankuchen have called attention to the fact that, as the rod-like particles of tobacco mosaic virus are brought closer and closer together in solution, the virus rods begin to assume positions parallel to each other even at distances of several hundreds of Å. This unusual phenomenon provides evidence for the existence of long

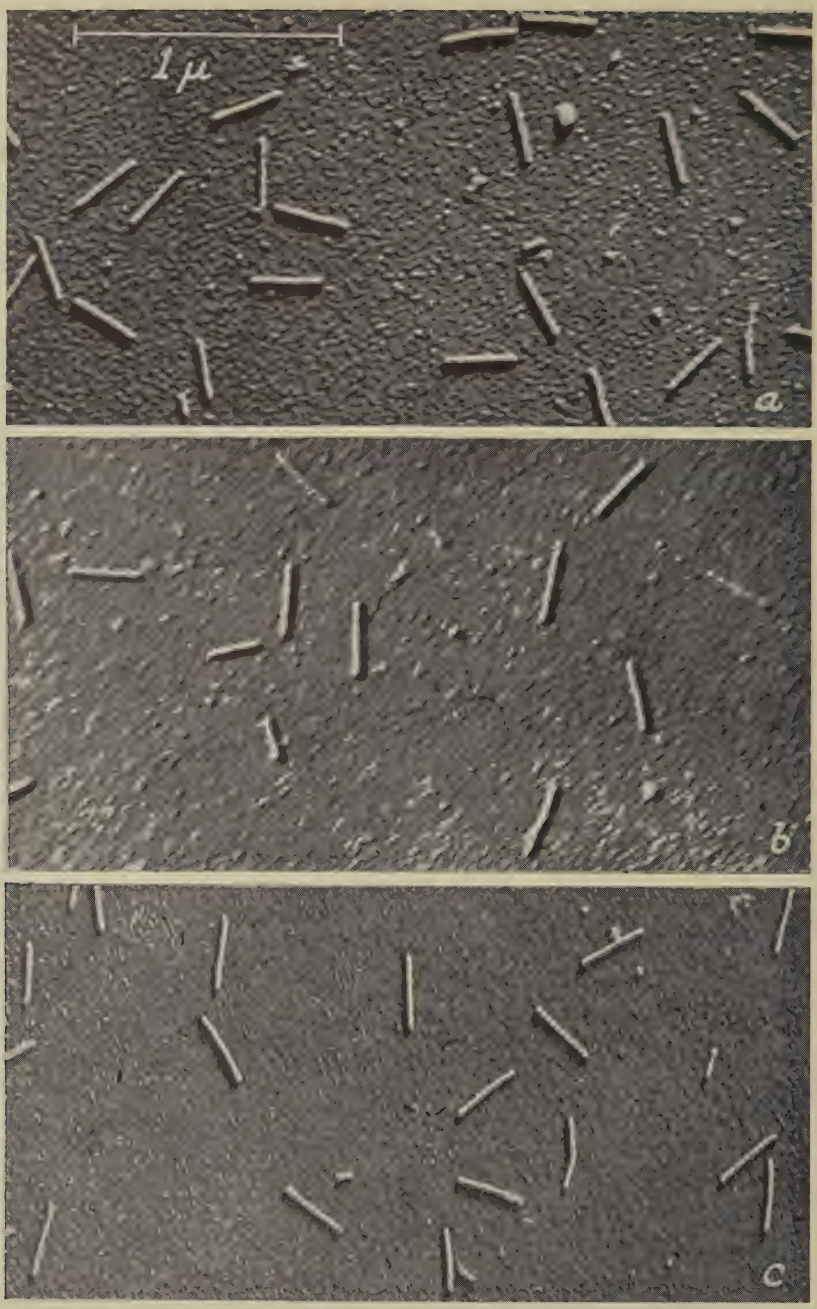
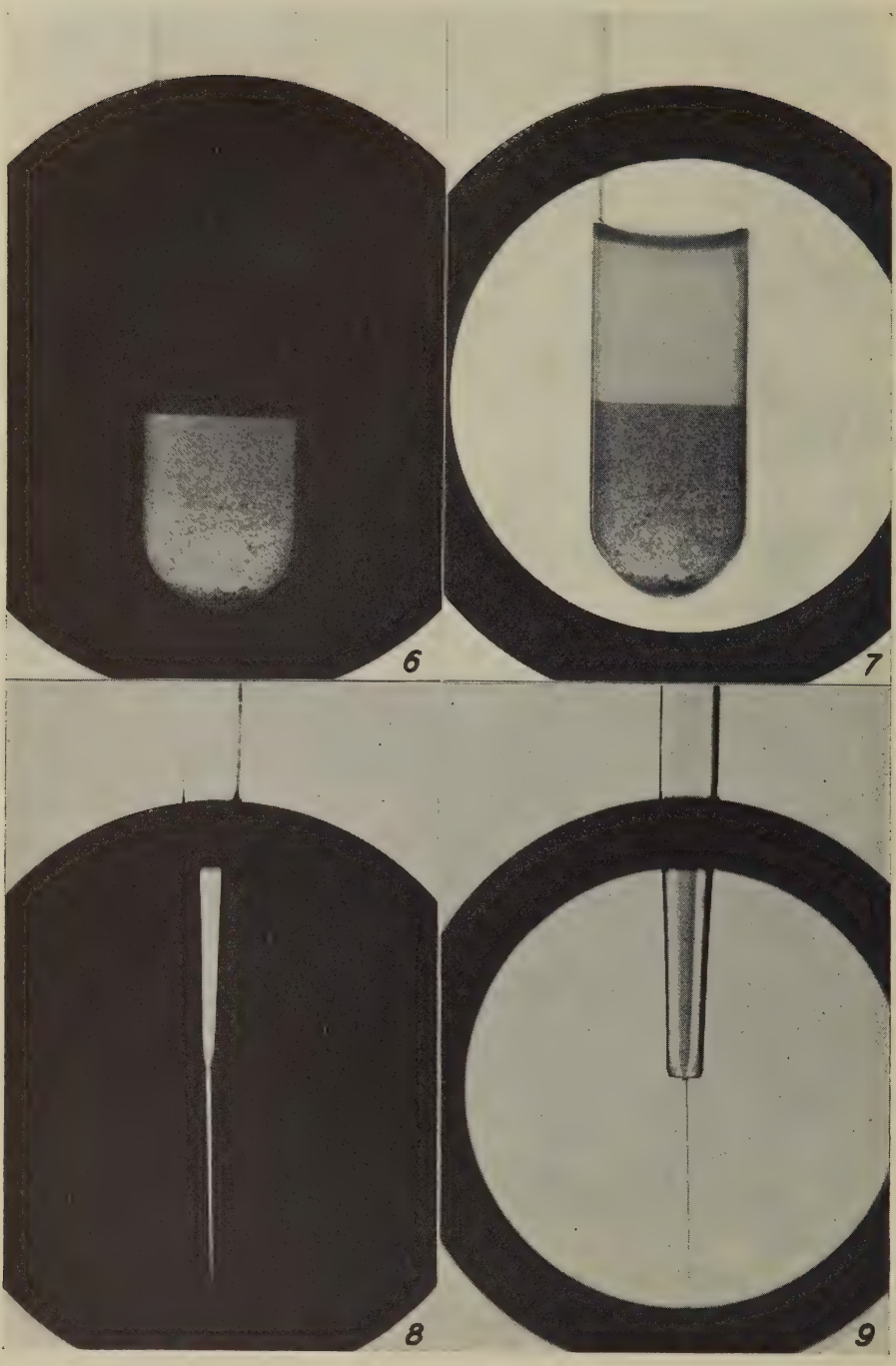


Fig. 5. Typical electron micrographs of the contents of hair cells from leaves of Turkish tobacco plants diseased with the (*a*) Dahlem, (*b*) Rothamsted, and (*c*) Princeton samples of tobacco mosaic virus. Mounts prepared with gold by the shadow-casting technique. (× 34,800). (From G. Oster, C. A. Knight and W. M. Stanley, *Arch. Biochem.*, 15 (1947) 279.)

range forces. These workers have also reported that the needle-shaped crystals of tobacco mosaic virus have no regularity in the direction of their length and hence, that the virus crystals have a two-dimensional, rather than a three-dimensional, type of regularity.

The size and rod-like shape of the particles of tobacco mosaic virus endow its solutions with some unusual properties. If a rather concentrated solution of purified tobacco mosaic virus is allowed to stand it will separate into two distinct layers. As can be seen from Figs. 6 and 7 the line of demarcation between the layers is quite sharp. The upper layer is not birefringent whereas the lower layer is spontaneously birefringent. The lower layer appears to consist of a three-dimensional mosaic of regions arranged at random to each other but in each of which all of the rod-shaped particles are orientated and are parallel to each other. The concentration of virus is somewhat greater in the lower layer than in the upper layer. Dilution of the lower layer yields a solution having the properties of the upper layer. When solutions of tobacco mosaic virus are caused to flow the flowing stream becomes birefringent, as shown in Figs. 8 and 9. This is due to the fact that the flowing stream causes the rod-like particles to line up parallel to each other and thus to assume a degree of orientation similar to that which obtains in a crystal. When an electric field is applied across a solution of tobacco mosaic virus either positive or negative birefringence may be shown. The electrical double refraction appears to be due to the orientation of the virus rods parallel to the field for the case of positive birefringence and perpendicular to the field for the case of negative birefringence. The double refraction of dilute solutions in an alternating field of 60 cycles appears to be positive for all values of field strength. The results indicate that the two ends of the virus rod differ. This difference may prove to be an important factor in the end-to-end aggregation of the rods of tobacco mosaic virus. When tobacco mosaic virus is dissolved in solvents having a refractive index approaching that of the virus, little or no stream double refraction is obtained. These and other studies indicate that the virus rods have little or no intrinsic double refraction. However, Bernal and Fankuchen have found, by means of X-ray studies, that the individual rods of tobacco mosaic virus have a regular inner structure of such a nature that each rod could be considered to be a crystal. The significance of this detailed structure within the rods of tobacco mosaic virus has not been elucidated. However, as described earlier, the entire rod appears necessary for virus activity, for breakage of the rod into two halves is accompanied by loss of virus activity. It may be of interest that heat treatment of



Figs. 6–9.

solutions of purified tobacco mosaic virus has yielded nucleic acid preparations having very large and asymmetric particles. The size and shape of these particles are such that it has been suggested that a virus rod has eight of these nucleic acid particles running the length of the virus rod. Perhaps it is this combination of amino acid and nucleic acid structure which yields the amazing virus activity. Needless to say, one of the important problems for the future is the elucidation of the nature of virus activity.

Much of the foregoing is a description of the isolation and properties of crystalline tobacco mosaic virus and includes the work on viruses which was recognized by the 1946 Nobel Prize for Chemistry. The isolation of the crystalline tobacco mosaic virus has been followed by very important work and, although there is not sufficient space to describe this subsequent work, it does seem fitting that the more important aspects should be mentioned here. Most viruses are known to exist in the form of different strains. Plants diseased with eight distinctive strains of tobacco mosaic virus were subjected to the same isolation procedure which yielded crystalline tobacco mosaic virus. The eight nucleoprotein preparations which were obtained were found to possess similar, yet distinctive properties. As can be seen from Fig. 10, the particles of the different virus strains were similar in size and shape. Of great importance was the fact that in a study of the amino acid composition of the purified preparations of the eight strains, definite differences, presumably

Fig. 6. A tube of tobacco mosaic virus solution which, upon standing, has separated into two layers. The photograph, taken with the aid of crossed Polaroid plates, shows that the bottom layer material is spontaneously doubly refracting, whereas the top layer material is not spontaneously doubly refracting. Double refraction of flow is shown by the virus in both layers. (From M. A. Lauffer and W. M. Stanley, *J. Biol. Chem.*, 123 (1938) 507.)

Fig. 7. The same system as in Fig. 6 taken between parallel Polaroid plates. The intensity of the light transmitted by the bottom layer is about the same as in the case represented in Fig. 6. (From M. A. Lauffer and W. M. Stanley, *J. Biol. Chem.*, 123 (1938) 507.)

Fig. 8. Doubly refracting stream of tobacco mosaic virus solution flowing from the end of a pipette, photographed between crossed Polaroid plates arranged so that each vibration direction of the Polaroid plates makes an angle of 45° with the direction of flow. (From M. A. Lauffer and W. M. Stanley, *J. Biol. Chem.*, 123 (1938) 507.)

Fig. 9. Same system as in Fig. 8 photographed between parallel Polaroid plates. The doubly refracting stream appears to be darker than the background in this case. (From M. A. Lauffer and W. M. Stanley, *J. Biol. Chem.*, 123 (1938) 507.)

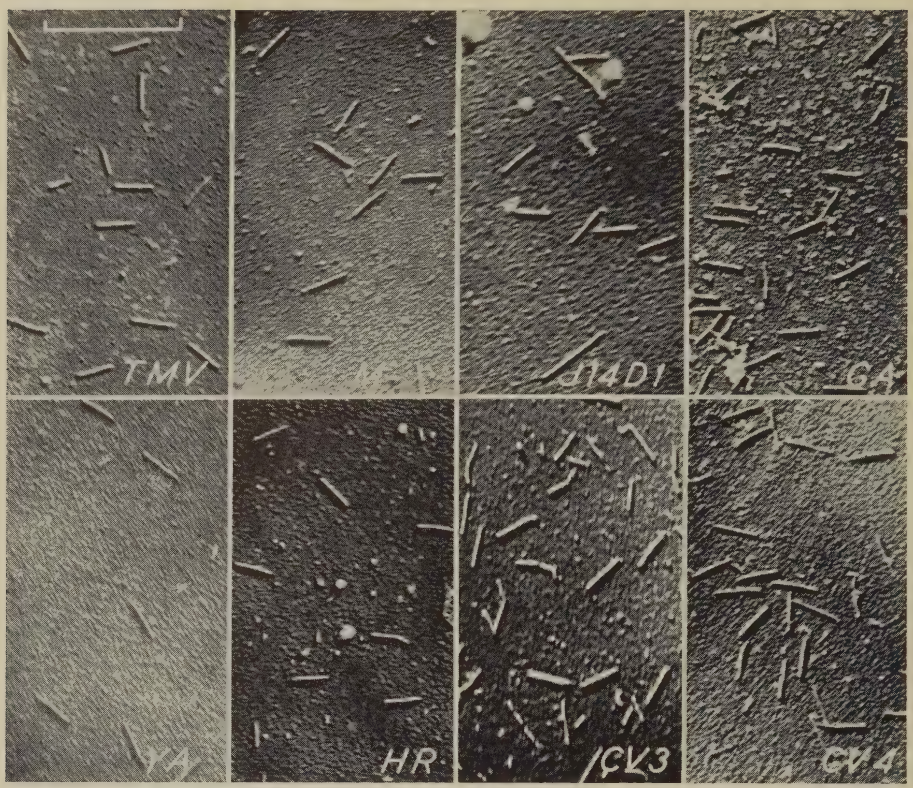


Fig. 10. Electron micrographs of 8 strains of tobacco mosaic virus. TMV–ordinary tobacco mosaic virus; M–Holmes' masked; J14D1–derivative of strain obtained by Jensen; GA–green aucuba; YA–yellow aucuba; HR–Holmes' rib-grass; CV3–cucumber virus 3; CV4–cucumber virus 4. The micrographs are of contents of hair cells from appropriately diseased Turkish tobacco plants except in the cases of CV3 and CV4, which were obtained from hair cells of diseased cucumber plants. Mounts prepared with gold by the shadow-casting technique. Line of the micrograph of TMV represents 1 μ. (From C. A. Knight and G. Oster, *Arch. Biochem.*, 15 (1947) 289.)

correlated with mutation of the virus, were found. The results, which are presented in Table 1, indicate that the mutation of a virus can be accompanied by the elimination of one or more amino acids from the virus structure, by the introduction of one or more amino acids to the virus structure or by a change in the concentration of one or more amino acids present in the virus structure. These results have great significance for biochemistry, for genetics and for medicine.

Chemical derivatives of tobacco mosaic virus possessing full virus activity

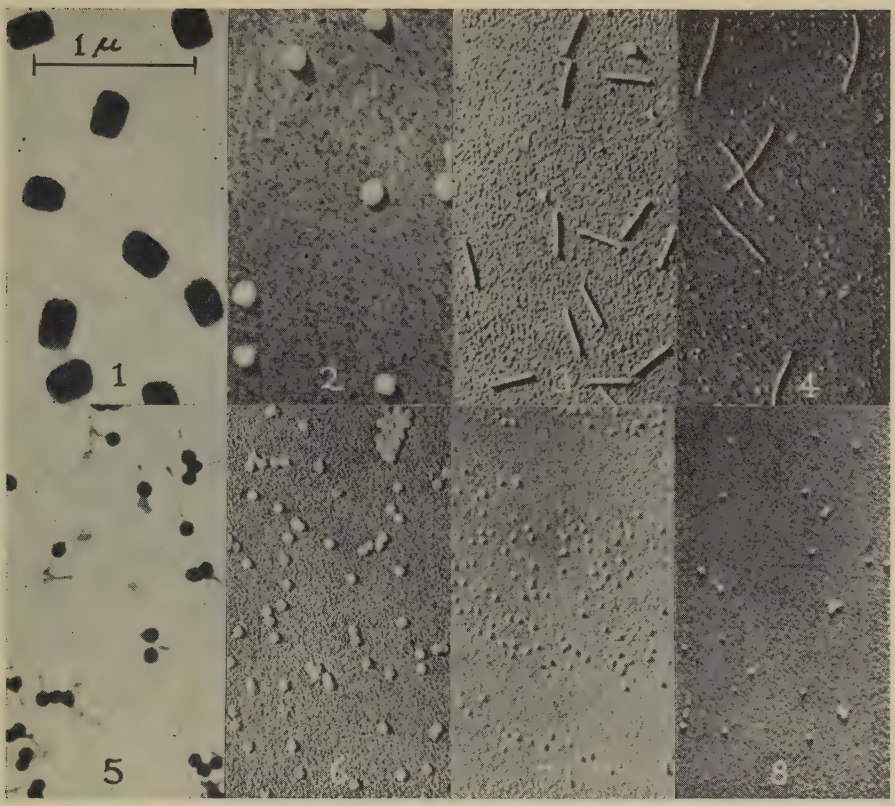


Fig. 11. Electron micrographs of purified virus preparations. All are at the same magnification and all except 1 and 5 were prepared by the gold shadow-casting technique. (*1*) Vaccinia virus; (*2*) Influenza virus (Lee strain); (*3*) Tobacco mosaic virus prepared from hair cells; (*4*) Potato X virus (latent mosaic of potato), hair cell preparation; (*5*) T_2 coli bacteriophage; (*6*) Shope rabbit papilloma virus; (*7*) Southern bean mosaic virus; (*8*) Tomato bushy stunt virus. (From C. A. Knight, *Symposia on Quantitative Biology, Biological Laboratory, Cold Spring Harbor, New York*, Vol. XII, 115–121, 1947.)

have been prepared. However, the virus produced by inoculation of the chemical derivatives has always proved to be ordinary tobacco mosaic virus. Work on the concentration and purification of different viruses has proceeded in several laboratories. Over a dozen viruses have been obtained in highly purified form, mainly by techniques involving high-speed centrifugation. Electron micrographs of the individual particles of eight of these purified virus preparations are shown in Fig. 11 at the same magnification. Because of the wide array of particle size and shape which they afford, purified virus

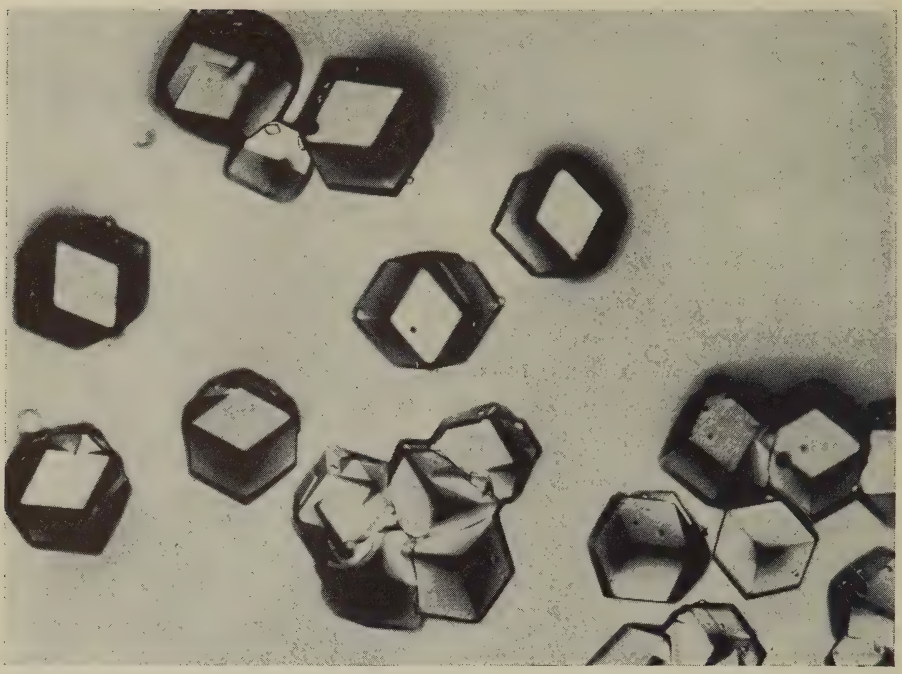


Fig. 12. Crystals of tomato bushy stunt virus (× 224). (From W. M. Stanley, *J. Biol. Chem.*, 135 (1940) 437.)

preparations are proving exceedingly valuable in connection with studies on particle interaction and other physicochemical phenomena. Some of these purified viruses are crystallizable nucleoproteins (Fig. 12), either rod-like or spherical in shape; others are nucleoproteins which have, as yet, not been crystallized, and others are large particles consisting of nucleoprotein, lipid, and carbohydrate, and possessing in some cases, a degree of morphological differentiation characteristic of organisms. Still other viruses have, as yet, defied isolation, possibly, in some cases, because of extreme instability. It is obvious that the chemical and physical properties of only a relatively few viruses have been determined. In view of the possibility that these represent the more stable and more readily purified viruses, one cannot be certain that a true picture of the chemical and physical properties of viruses as a whole has been obtained as yet.

The new field of virus research is really in its infancy and much remains to be accomplished. Certain basic and fundamental problems relating to the mode of virus reproduction and mutation have taken definite form. Solution of these problems should yield information of great value to biology, chem-

istry, genetics and medicine. This new field of research has been laid upon a very broad foundation, for the present status of virus research has been achieved by contributions from almost every branch of science. It is likely that a continuation and extension of the application of weapons from many fields of science will be necessary for the proper and full development of the new field of virus research.

Biography

Wendell Meredith Stanley was born in Ridgeville, Indiana, on August 16th, 1904. He began his advanced education at Earlham College and graduated Bachelor of Science in 1926 when he entered the University of Illinois, gaining a Master of Science degree in 1927 and a Ph. D. in chemistry in 1929. He continued at Illinois as a research associate and later as an instructor before leaving, in the latter half of 1930, to do research at Munich as a National Research Council Fellow. In Munich he worked with Heinrich Wieland until late 1931 when he returned to the United States to take up a post as Assistant at the Rockefeller Institute. He remained with the Institute until 1948, becoming an Associate Member in 1937, and a Member in 1940. In 1948, he was appointed Professor of Biochemistry and Director of the Virus Laboratory, University of California; during 1948–1953 he was Chairman of the Department of Biochemistry, and in 1958 he became Professor of Virology and Chairman of the Department.

Stanley has been responsible for much important work on lepracidal compounds, diphenyl stereochemistry and the chemistry of the sterols. His researches on the virus which causes the mosaic disease in tobacco plants led to the isolation of a nucleoprotein which displayed tobacco mosaic virus activity. The virus appeared to act like an inanimate chemical but it presented evidence of being a living and growing organism. His more recent work on the preparation and investigation of influenza and similar viruses has led to his development of the centrifuge-type influenza vaccine. He is a recognized world authority on viruses and, as such, he has written over 150 papers on the topic and contributed chapters to several books.

Professor Stanley was awarded the American Association for the Advancement of Science Prize in 1937, and his many other honours and awards include the Rosenburger Medal (University of Chicago), Alder Prize (Harvard), and Scott Award (City of Philadelphia), 1938; Gold Medal of the American Institute of New York, 1941; Copernican Citation, 1943; Nichols Medal (American Chemical Society), 1946; Gibbs Medal (American Chemical Society), 1947; Franklin Medal and Presidential Certificate of Merit,

1948; the Modern Medicine Award, 1958; and the American Cancer Society's Medal for Distinguished Service in Cancer Control, 1963. He has been awarded honorary Doctor of Science degrees by many universities and colleges, including Earlham, Harvard, Yale (1938), Princeton (1947) and Illinois (1959); honorary Doctor of Law degrees of the Universities of California (1946) and Indiana (1951), the Jewish Theological Seminary of America (1953) and Mills College (1960); and Doctor, *honoris causa*, University of Paris (1947).

He has served on the Councils of many academic, medical and other learned societies, and as consultant and advisor to the United States government and the World Health Organization. He continues as Director-at-large, American Cancer Society and as a Member of the Board of Scientific Counsellors of the National Cancer Institute. He is a member of many scientific societies.

Wendell M. Stanley married Marian Staples on June 15th, 1929. They have one son, Wendell M. Junior, and three daughters, Marjorie Jean, Dorothy Claire, and Janet Elizabeth.

Chemistry 1947

Sir ROBERT ROBINSON

«for his investigations on plant products of biological importance, especially the alkaloids»

Chemistry 1947

Presentation Speech by Professor A. Fredga, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesty, Royal Highnesses, Ladies and Gentlemen.

One of the principal aims of organic chemistry is to make clear the chemical structure of substances found in living nature. Interest has been directed particularly towards substances with vital functions or otherwise obvious qualities. The structure of simpler compounds was largely elucidated during the nineteenth century, the more complicated ones being reserved for our century. Sir Robert Robinson's exceedingly fruitful work treats many groups of such substances. In comprehensive investigations he has dealt with the anthocyans, a group of red, blue, or violet pigments found almost everywhere within the vegetable kingdom, and which we meet with in the cornflower and the lark-spur of the fields as well as in claret and beetroot. He has done important work on sex hormones and synthetic substances of less complicated structure but with similar properties. He has done pioneering work on synthetic drugs against malaria, he has contributed towards the investigation of penicillin and he has successfully attacked fundamental questions concerning the mechanism of organic-chemical reactions. In presenting him with this year's Nobel Prize in Chemistry, the Royal Academy of Sciences has in mind, however, particularly his work on alkaloids.

By alkaloids we understand a numerous group of nitrogenous basic substances from the vegetable kingdom. They usually have striking, sometimes sensational physiological effects. Among them are quinine, cocaine, and atropine, all of which have important medicinal qualities, further morphine, doubtless well-known, and strychnine known for its medicinal value and – in somewhat larger doses – as an exceedingly active poison. Plants containing alkaloids have generally drawn the attention of primitive peoples, and in the cases where they are met with in countries with ancient culture, the knowledge of their properties often goes back to pre-historic age. They have been used as medicines and means of enjoyment, for ritual and criminal purposes. They can carry our thoughts to poetry and romance – it is not only decadent poets who have sung the praise of opium and poppy juice – but they have also been associated with vice, crime and horror.

During the nineteenth century we began to learn how to isolate the active substances themselves, the alkaloids, and investigation of their chemistry still continues with unabated interest. It was soon found that these alkaloids are usually very complicated in structure; the molecule of morphine contains 40 atoms, that of strychnine 47, each of which has its definite place in relation to the others. To reveal the inner architecture of these complicated systems through different chemical operations is a task as difficult as it is fascinating. It requires great experimental skill, creative power and sharp logic. In this sphere of alkaloid research, Sir Robert stands out as our foremost contemporary. He has solved the riddle of the morphine molecule's structure, in connection with which quite 20 different formulae have been under consideration, he has clarified the essential features of the strychnine formula, even though some details are still uncertain, and he has made decisive contributions towards the investigation of many other alkaloids with strangely sounding names like gnoscopine, harmaline, physostigmine, and rutaecarpine.

It has often been asked how plants build up these singular molecules. Here, Sir Robert has formed a theory which rests upon the amino-acids contained in proteins, and which seems to present a satisfactory answer to the question. The theory is illustrated by Sir Robert's famous synthesis of tropinone, a substance closely related to cocaine. We have here a case where three rather simple molecules spontaneously unite into a complicated system, which earlier we could only build up step by step through a long series of reactions. We may suppose that here Sir Robert has found the key to nature's own way of working. This theory has also gained great importance as a guide when determining intricate structures, and it has rendered it possible to trace hidden connections within the multifarious group of alkaloidal substances.

The tendency in natural science tends more and more to the removal of the traditional boundaries between the different sciences. The sum of total knowledge constantly increases, human intellect, however, is limited and co-operation therefore becomes a matter of necessity. For the individual scientist it becomes a difficult task to broaden and deepen his science on its own particular basis without turning his back upon productive collaboration. Perhaps this is felt particularly in chemistry; it is there that the threads of research into life and matter run together, and thus chemistry has acquired a key position within the natural science of to-day. Sir Robert has solved the problem with great success. He has devoted his life to organic chemistry, but the importance and the consequences of his work extend far into the fields of biological and medicinal research.

Professor Sir Robert Robinson. The intricate problems of organic structure are not of a nature to attract the interest of the general public. Our science is an exclusive one. You have not gained your scientific reputation by startling discoveries, which, like the atomic fission, resound in the columns of the daily press.

By your very important and very numerous investigations, you have gradually changed our ideas on fundamental questions. As a student of molecular architecture you have, with eminent success, pursued the line of work emerging from Kekulé and Couper, and you have thrown light upon the formation of complicated structures within the living plant. Among organic chemists, you are to-day acknowledged as a leader and a teacher, second to none. In recognition of your services to Science, the Royal Academy has decided to bestow upon you the Nobel Prize for Chemistry for your investigations on plant products of biological importance and especially for your outstanding work on the structure and the biogenesis of complicated alkaloids.

Sir Robert. On behalf of the Academy, I request you to receive your prize from the hands of His Majesty the King.

Robert Robinson

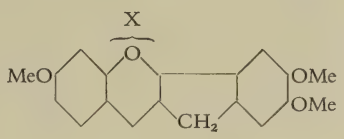
Some polycyclic natural products

Nobel Lecture, December 12, 1947

In dealing with this subject I do not propose to attempt any full description of a part of my researches in the fields of the alkaloids, plant colouring matters, or on the methods of synthesis of steroids, but rather to indicate some connecting threads and perhaps, in some places, to go a little behind the scenes. In 1905, I was privileged to be given a place in the private laboratory of my revered teacher, Professor W. H. Perkin, Jr. at the University of Manchester. The synthesis of limonene had just been completed and sylvestrene was the next objective. Such work involved long and laborious preparations and would not have suited my case. It was indeed a fortunate chance that Perkin introduced me instead to the catechol derivatives which, together with their cousins of the *meta* and *para* series, have befriended so many organic chemists, myself among them, by reason of the numerous colour reactions that they exhibit and by the versatility of their transformations. Perkin's idea was to prepare a series of hydroxylated coumarins from ethyl piperonoylacetate and to study their dyeing properties. « My brother Arthur », he said, « is a dabhand with natural dyes and will help us ». I made the desired ester, by adapting Claisen's elegant method for ethyl benzoylacetate, but not the coumarins, because of the interest that I very soon took in the brazilin problem. At that time experimental study was concentrated on brazilein and its derivatives. This was in the hands of one Johann Engels, supposed by the students to be a retired manufacturer working for his own satisfaction; he inhabited some subterranean cavern of the laboratory but emerged from time to time bringing gifts of most beautiful orange and crimson crystals to the private laboratory.

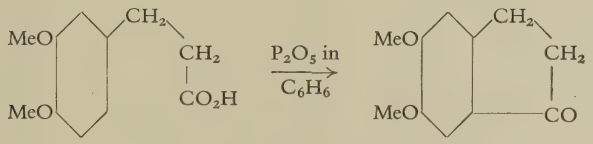
It seemed that the materials in my hands could be used in the brazilin investigation and I sought and obtained permission to turn in that direction. We soon encountered highly characteristic methylated brazylium salts, related to the isobrazilein sulphate of Hummel and A. G. Perkin (1882) and on the basis of the current brazilin structure of Perkin these were formulated as (I). There was no known method that could be applied to the synthesis of (I), except, perhaps, that of Bülow. Attempts to devise a new process led to the

discovery of the condensation between salicylaldehydes and keto-methylenes in the presence of hydrogen chloride. This synthesis of pyrylium salts was independently and simultaneously described by Decker and Fellenberg (1907).



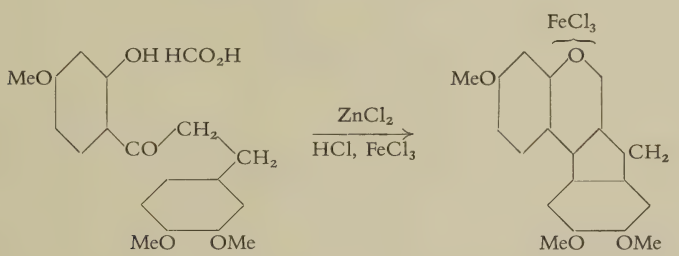
(I)

Applying it, *p*-methoxy-salicylaldehyde and 5:6-dimethoxy-α-hydrindone (II) gave a chloride (and ferrichloride) corresponding to (I) and these salts were not identical with the isomerides derived from brazilin.



(II)

The accepted constitution of brazilin was established by syntheses of anhydrobrazilic acid and brazilinic acid (1908), deoxytrimethylbrazilone and trimethylbrazilone (1926–1928; parallel work by P. Pfeiffer), trimethoxybrazylium salts (with H. G. Crabtree, 1918) and certain of the hæmatoxylin analogues. The first synthesis of a substance containing the ring skeleton was that of trimethoxybrazylium ferrichloride (III).

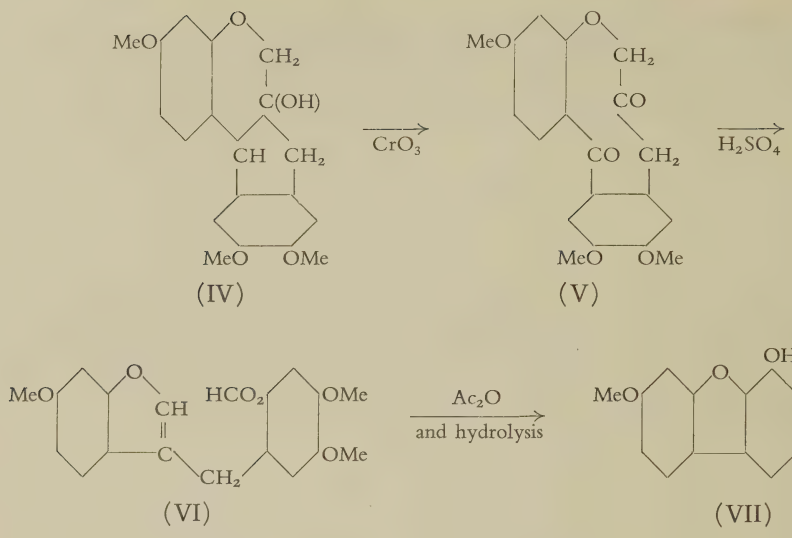


(III)

The manifold and intriguing transformations of the brazilin molecule, particularly those of trimethylbrazilone (V), may now be said to be well understood but synthetical confirmation has not always been obtained.

Only this year my collaborator, K. W. Bentley, synthesized β-anhydrotrimethylbrazilone (VII) obtained from trimethylbrazilin (IV) by the stages (V) and (VI).

The study of brazilin and hæmatoxylin is by no means finished and in this



connexion I would like to make a general observation. The synthesis of brazilin would have no industrial value; its biological importance is problematical, but it is worth while to attempt it for the sufficient reason that we have no idea how to accomplish the task. There is a close analogy between organic chemistry in its relation to biochemistry and pure mathematics in its relation to physics. In both disciplines it is in the course of attack of the most difficult problems, without consideration of eventual applications, that new fundamental knowledge is most certainly garnered.

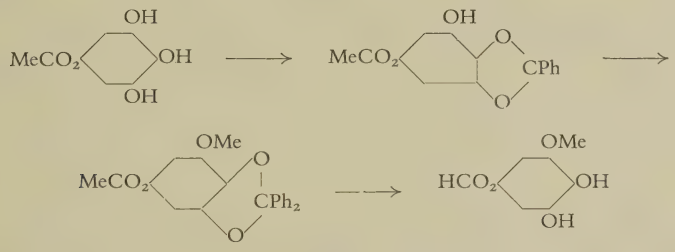
From the incidents of the brazilin investigation two fresh topics developed. In the first place the pyrylium salt synthesis led to the much later extension to the synthesis of anthocyanidins and still later to that of the anthocyanins (see below). Secondly the facile ring closure of β-3 :4-dimethoxyphenylpropionic acid to the ketone (II) suggested an application to the synthesis of papaverine. That was actually accomplished but not published owing to anticipation by Pictet and Gams (1909). Otto Wallach was in Manchester for a few days at that time and advised publication nevertheless. We did not act on this advice because our final intermediate was an oil and Pictet and Gams had crystallized it. Thirty years later I found that these crystals do not consist of the required compound, and Pictet and Gams must also have used the oil for the synthesis of papaverine.

A side-issue was that, as a small consolation for this disappointment, a new oxazole synthesis was developed by dehydration of the acylamino-ketones, prepared as models in the papaverine work. It was natural also to turn to

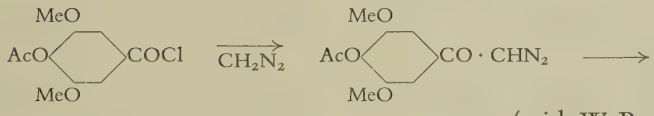
other members of the isoquinoline group of the alkaloids and this led in stages to the syntheses of narcotine, hydrastine and berberine, and to a general wider concern with alkaloid chemistry.

Some further connexions between apparently unrelated investigations are mentioned below although there have certainly been several fresh points of departure. Electronic theories of chemical reactions are not part of my present topic but it may be recalled that the condensation reactions of pseudo bases (carbinol-amines) and the C-alkylation of unsaturated amines were among the experimental foundations of my theoretical system and they arose from early work on alkaloids.

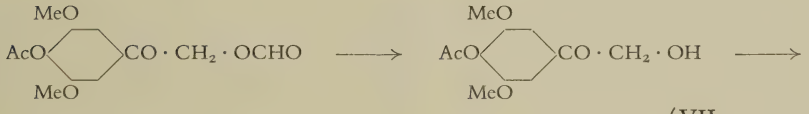
Anthocyanins. The general pyrylium salt synthesis, mentioned already, was found (with D.D.Pratt, 1922 etc.) to be applicable to the synthesis of the anthocyanidins. Then (with A.Robertson, 1926 etc.) it was first shown that glucosidylflavylium salts could be prepared and then some of the simpler anthocyanins were synthesized. It became chiefly a question of preparation of the necessary intermediates and this depended largely on the use of appropriate protecting groups. The following illustrates some special devices:



(with W. Bradley and G. Schwarzenbach, 1930)



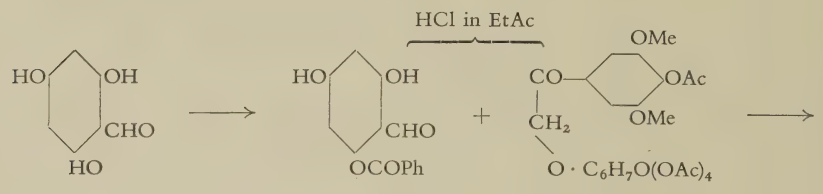
(with W. Bradley, 1928)



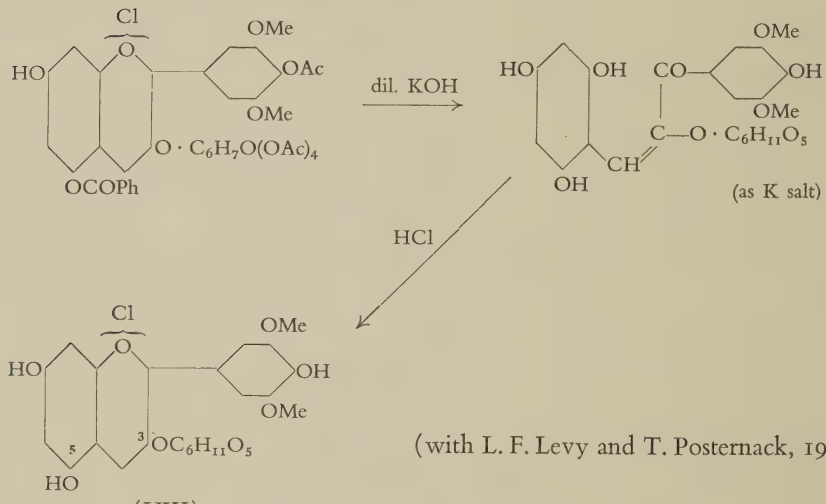
(VII, see p. 170)

The formation of diazoketones from acid chlorides and diazomethane was first observed in the course of these investigations and found to be of great service.

As the work proceeded the effort was made to reduce the protection of hydroxyl groups to the minimum and, rather as a *tour de force*, it was shown that cyanin could be synthesized even by use of unprotected components. In most cases, monobenzoylphloroglucinaldehyde was an effective starting point and if a sugar residue was required in the phloroglucinol nucleus that was adequate protection in itself. The following scheme, representing the synthesis of oenin chloride (VIII), is an illustrative example.



(VII)



(VIII)

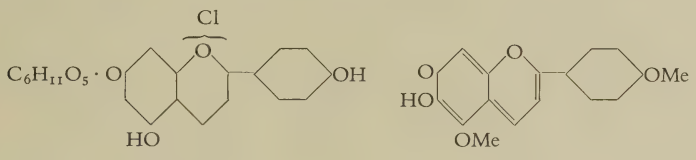
(with L. F. Levy and T. Posternack, 1931)

The important 3:5-diglucosides were synthesized in a similar fashion (with A. R. Todd, 1932) from the 2-tetra-acetylglucoside of phloroglucinaldehyde. This was fortunately obtained by direct aceto-glucosidation in acetonitrile solution.

Possession of the pure synthetic specimens of the anthocyanidins and chief anthocyanins enabled my wife and me to devise quick tests for these colouring matters which can be used with the material from a few flower petals. Hence it was possible to make a survey of anthocyanins. As a result one quite

new type was discovered in the flowers of *Gesnera fulgens*. This proved to be an apigeninidin glucoside (IX) which was synthesized for comparison.

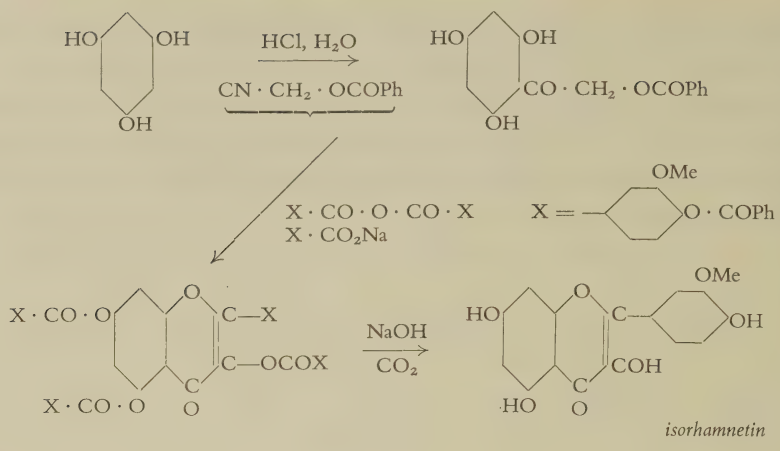
The cosmetic pigment *carajura* formerly made by Indians of the Orinoco basin was found to contain crystallizable carajurin (X) (with E. Chapman and A. G. Perkin, 1927) which is the base of a somewhat similarly constituted flavylium salt.



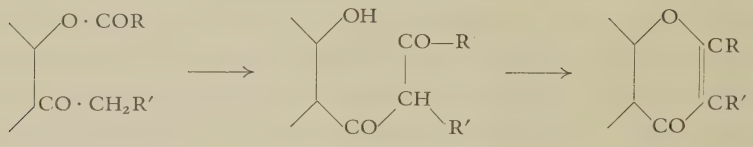
(IX) (X)

New anthocyanins, not yet identified, occur in the young fronds of certain ferns (with J. R. Price, V. C. Sturgess and G. M. Robinson, 1938) and other unsolved problems are those of the nitrogenous anthocyanins such as the pigments of beet and of bougainvillaea, as well as the curious yellow colouring matter of the Iceland Poppy (with J. R. Price and R. Scott-Moncrieff, 1939). It is suspected that amino-acid residues occur in these compounds. The rapid tests which we employed were found to be serviceable in genetic investigations of flower colour. They can now be supplemented by paper chromatography and a promising field of investigation in plant biochemistry is thus opened up.

Only a brief reference can be made to the related subject of the anthoxanthins of the flavone and flavonol classes. Since partly methylated anthocyanidins could be synthesized so easily, it seemed desirable to acquire parallel powers in the more oxidized series and thus an entirely new method had to be devised. It was clear that the final stages would need to be more gentle than is prolonged boiling with concentrated hydriodic acid. The method eventually developed was the aroylation of *o*-hydroxyacetophenones by means of an acid anhydride, and the hydrolysis of the acylated product. This process was applied in my laboratory to the synthesis of chrysin, apigenin, acacetin, luteolin, diosmetin, galangin, kaempferol, datiscetin, fisetin, quercetin, morin, myricetin, gossypetin and quercetagetin; it has become the standard flavone synthesis and has been considerably employed by other chemists. The synthesis of isorhamnetin (with T. Heap, 1926), a methyl ether of quercetin occurring in yellow Wallflowers, may be given in illustration.



W. Baker has shown that the aroylation proceeds in two stages and involves a migration from O to C.



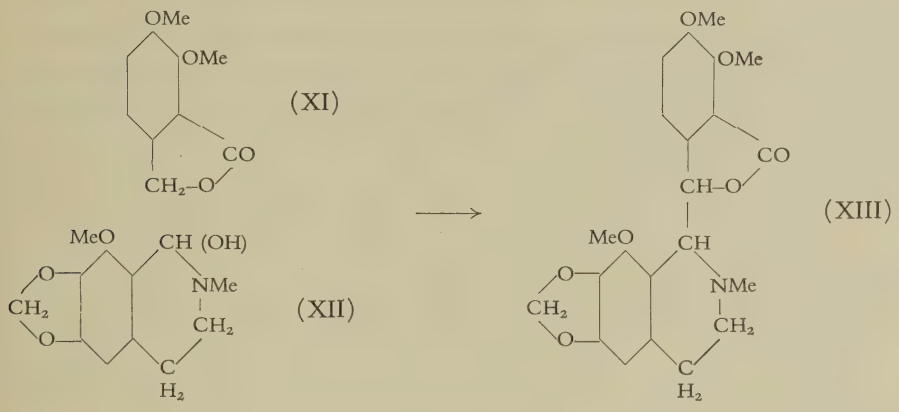
The synthesis of isoflavones, the conversion of catechin into cyanidin, and work on leuco-anthocyanins, cyanomaclurin and peltogynol were by-products of the studies of plant pigments.

I would like to add that no weed crops up in gardens more persistently and regularly than does the idea in the chemical literature that anthocyanins are formed in Nature from anthoxanthins by reduction (or *vice versa* by oxidation). There is no direct evidence that this is so; the statistical evidence opposes the theory, and the genetic studies of Dahlia by Lawrence and Scott-Moncrieff give clear indication that the flavones and flavylium compounds are formed by divergent processes, possibly from the same starting point, already, however, considerably elaborated as compared with the primary carbohydrate material.

Alkaloid studies. After the papaverine incident it was decided to attempt the synthesis of narcotine (XIII) which could be a condensation product of cotarnine (XII) with meconine (XI). Cotarnine was thought of as an aldehyde and meconine as a keto-methylene compound —CO—C=C—CH_2— and we knew that Lapworth had shown the methyl of similarly constituted ethyl

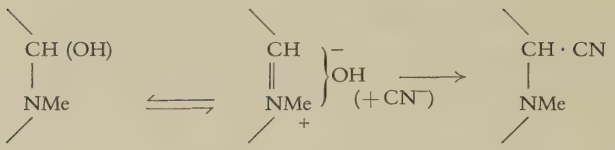
crotonate to be reactive; for example, it could be oxalylated. Therefore an alkaline catalyst was used, potassium carbonate was chosen in order to avoid hydrolysis of the lactone. The reaction gave a small yield of *dl*-narcotine (α-gnoscopine) in boiling alcohol and this racemic substance was resolved, with some difficulty, into *d*- and *l*-narcotine (natural).

The yield was improved by employing halogenated meconines and on dehalogenation, α-gnoscopine was obtained. Condensation of cotarnine with

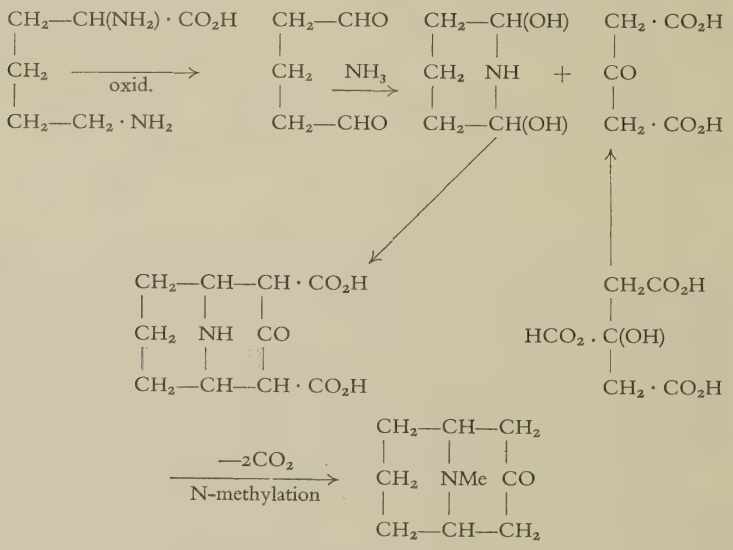


nitro-meconine afforded a nearly quantitative yield of a base recognized as nitro-β-gnoscopine. On removal of the nitro-group ($NO_2 \rightarrow NH_2 \rightarrow NH \cdot NH_2 \rightarrow H$; $NH_2 \rightarrow I \rightarrow H$) β-gnoscopine was produced. This could not be resolved but it was synthesized from *d*- and *l*-β-narcotines produced by partial racemization of the *d*- and *l*-narcotines. I will not pursue this topic except to mention that hydrastine and bicuculline have been synthesized in similar fashion. Hydrastine does not conform with narcotine in stereochemical configuration.

I have mentioned these researches chiefly because they introduced me to the facile condensation reactions of pseudo-bases, the carbinol-amines, of which cotarnine is very typical. The earlier work of Liebermann, and of Kropf, was greatly extended (with E. Hope, 1913). Cotarnine condenses very readily with alcohols, mercaptans, amides, ketones such as acetone and acetophenone, malonic ester and the like, phenylacetonitrile, indene, fluorene, α-methylindole, nitromethane, 2:4-dinitrotoluene, etc., etc. The reactive form is now considered to be the ammonium hydroxide and a typical case is represented by the formation of cyanodihydrocotarnine. The part formulae are:



It was impossible not to be impressed with the smooth progress of such reactions and the conviction came that they must be of importance in Nature, especially since carbinol-amines can be regarded as oxidation products of amino-acids. These ideas germinated while still at the University of Manchester and developed during the period of tenure of my first Chair of Chemistry at Sydney, New South Wales. There I wrote down a possible biosynthesis of pseudopelletierine (XIV) from lysine and citric acid, based on pseudo-base condensations.

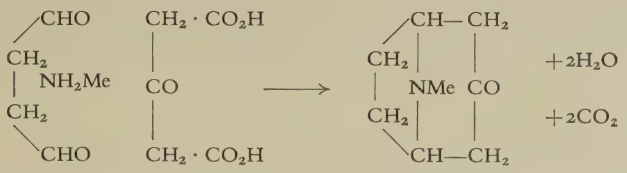


(XIV)

In the lower ring-homologous series the position of the carboxyl group of ecgonine seemed to give support to the hypothesis. On returning to England the opportunity arose at the University of Liverpool to test the scheme experimentally. We were in the midst of the First Great War and a shortage of atropine was threatened.

I found that succindialdehyde, methylamine, and acetonedicarboxylic acid or its calcium salt, mixed in aqueous solution at the ordinary temperature, gave a notable yield of tropinone (XV) after acidification. C. Schöpf has

since shown that at so-called physiological pH the base is directly produced in very high yield.

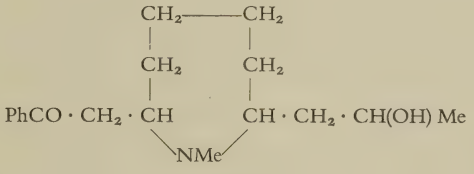


(XV)

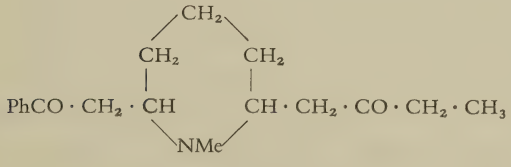
Other applications of this method of construction of bicyclic bases were made by replacing succindialdehyde by glutardialdehyde (pseudopelletierine) (with R. Menzies, 1924), adipdialdehyde, levulinaldehyde, thiodiacetaldehyde (also NMe $(CH_2 \cdot CHO)_2$ and Se $(CH_2 \cdot CHO)_2$ with B. K. Blount, 1932); the acetonedicarboxylic acid may be replaced by its semi-ester (Willstätter) so as to provide a synthesis of a stereoisomeride of ecgonine.

The hypothesis that the tropine group is derived from ornithine, or that both are derived from a common parent, was supported by Willstätter's recognition of $\alpha\delta$-tetramethyldiaminobutane among the coca bases, also proline, hygrine, and cuschygrine readily fell into line.

In 1917 I drew attention to certain structural relations of the alkaloids, many of which seem significant to-day, though after thirty years the paper needs to be re-written in detail. The pyrrolidine group was related to ornithine and a C_3-fragment, the acetone equivalent. The piperidine group was similarly related to lysine and the C_3-fragment. The existence of a still higher homologous group is just indicated by Wieland's studies of the alkaloids of lobelia (1921 etc.) because lobinine is supposed to be (XVI).

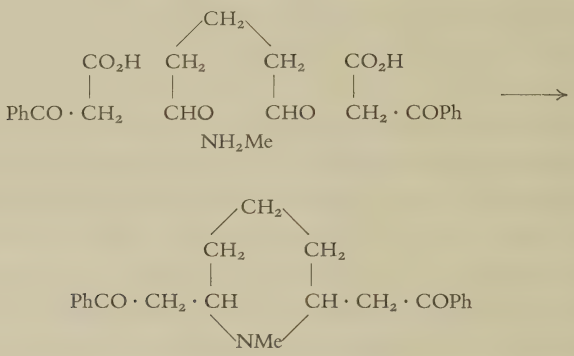


(XVI)



(XVII)

However the other alkaloids of lobelia are clearly members of the lysine group and C. Schöpf (1929) synthesized lobelanine (XVIII) by an adaption of the pseudopelletierine method. Glutardialdehyde, benzoylacetic acid, and methylamine, mixed together in buffered solution at pH 4.5, furnished the alkaloid.



(XVIII)

The exceptional case of lobinine should be re-investigated; the evidence does not exclude the structure (XVII) and an additional carbon atom in the C_3-fragment presents no serious difficulty.

The structural or biogenetic relations of plant products as deduced from the recognizable architectural components of the molecules have been consistent guides in my investigations. An early instance was the change proposed in the constitution of berberine (1910). It seemed highly improbable that hydrastine and berberine, congeners in *Hydrastis canadensis*, could have totally differently oriented methoxyl groups. The change suggested was justified by the first synthesis of berberine by way of oxyberberine (with Perkin and Ray, 1925). Similar considerations underlay the revision of the structures of morphine and its relatives, made with J. M. Gulland in 1923–1924. As a recent development of this topic was the subject of my Presidential Address to the Royal Society a few days ago, I will not pursue it here.

But the most fruitful thought along these lines was that which led to the establishment of an indole group of the alkaloids (with Perkin and Kermack).

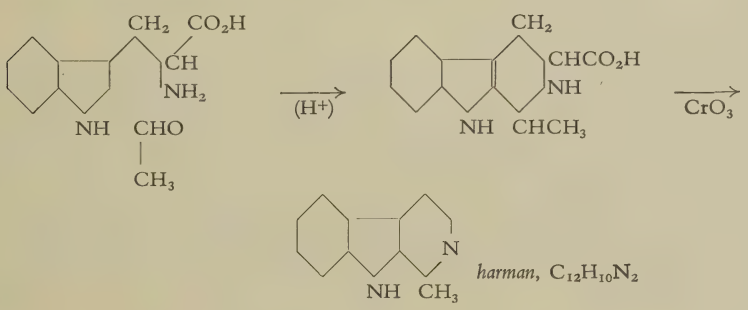
Here I remember very clearly the sequence of events and must admit that this was obscured in the publication. Otto Fischer had degraded harmine, $C_{12}H_9N_2 \cdot OCH_3$, to harman, $C_{12}H_{10}N_2$, and the first clue arose from writing down the possible, or at least the most likely, structures for this sub-

stance. A great many of them would be methyl derivatives, $C_{11}H_7N_2 \cdot CH_3$, of $C_{11}H_8N_2$ which is the formula of a tricyclic aromatic substance containing benzene, pyrrole, and pyridine nuclei. Hence in 1912 we sought, and found, this methyl group, and also eliminated it. This was made easy by the fact that harmine, like quinaldine, forms a benzylidene derivative:

$$CH_3O \cdot C_{11}H_6N_2 \cdot CH_3 \rightarrow CH_3O \cdot C_{11}H_6N_2 \cdot CH{=}CHPh \rightarrow$$
$$CH_3O \cdot C_{11}H_6N_2 \cdot CO_2H \rightarrow CH_3O \cdot C_{11}H_6N_2 \text{ (norharmine).}$$

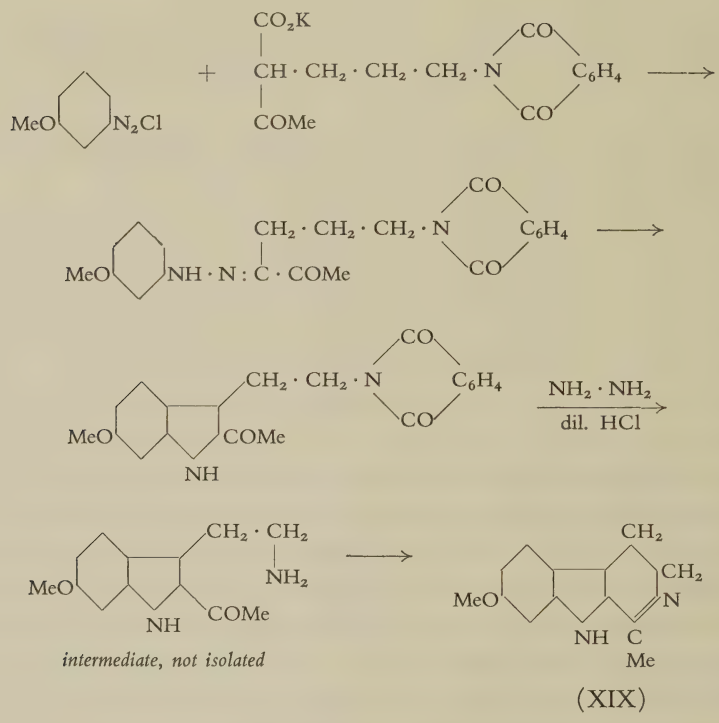
Later, norharman, $C_{11}H_8N_2$, was prepared by removing the methoxyl group from norharmine or the methyl group from harman.

It seems incredible that the next step was not taken until some years had elapsed. One day, when in Liverpool, I happened to wish to refer to Richter's Lexicon for the melting point of a methylnaphthiminazole, $C_{12}H_{10}N_2$. It was interesting to see an old friend, harman, as No. 16 and a little further I noticed (No. 19) Verbindung (aus Tryptophan) and a Zentralblatt reference of 1903. The paucity of the information and the coincidence induced me to read the original (Hopkins and Cole, 1902). The base from tryptophan had m.p. 238° which was a few degrees higher than that attributed to harman but otherwise the properties of the two substances, especially the characteristic fluorescence in acid solution, were identical. Later the m.p. of harman was raised to 238° and direct comparison with a specimen of Hopkins' base proved the identity. The mystery of the formation of a C_{12} base by oxidation of tryptophan (C_{11} and loss of CO_2H occurs) with aqueous ferric chloride was easily resolved. The mixture had been extracted with ether and left for a few days; there is little doubt that alcohol in the ether was a source of acetaldehyde. It was in fact possible to synthesize harman by condensation of tryptophan with acetaldehyde followed by oxidation, or by joint oxidation of tryptophan and alanine.



Harman was later found by Späth to be identical with the alkaloid aribine.

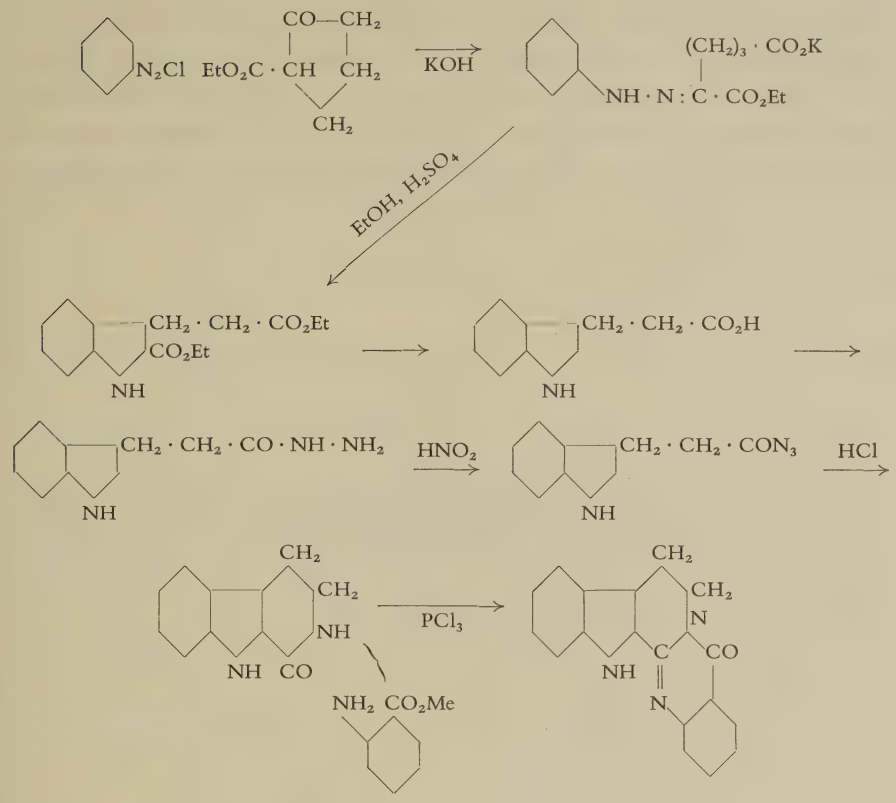
Numerous syntheses of harmine and its analogues and degradation products have been effected and perhaps the most interesting is that of the alkaloid harmaline (dihydroharmine) (XIX) carried out by R. H. F. Manske in my laboratory at Manchester (1927).



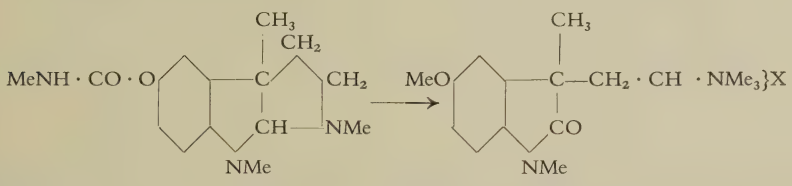
(XIX)

This was the first example of the use of hydrazine for the hydrolysis of a phthalimide. The rapid expansion of the indole group of the alkaloids was phenomenal. Our norharman was identified by Asahina with a degradation product of evodiamine and in collaboration we synthesized rutaecarpine (XX) (with R. H. F. Manske).

By analogy with the C-methylation that evidently leads to corydaline in Nature, and with the laboratory β-C-methylation of indoles, an angle-methyl formula based on a hydroxytryptophan was suggested for eserine (cf. E. Stedman and G. Barger, *J. Chem. Soc.*, 127 (1925) 248). This view was first synthetically confirmed by the synthesis and resolution of the quaternary salt (XXI) which can also be obtained from eserine (with H. Suginome and F. E. King, 1932–1935)

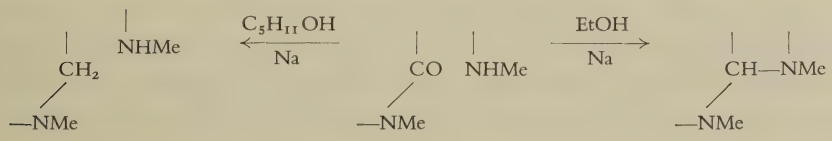


(XX)

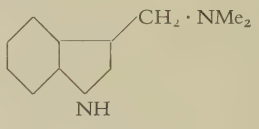


(XXI)

The complete synthesis of eserine was accomplished by Julian and Pikl (1925). We missed it because we used sodium and amyl alcohol for a reduction which was carried beyond the desired stage.

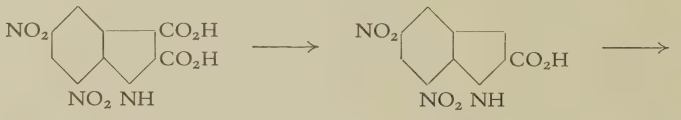


The indole group now includes many types varying from the remarkably simple gramine (donaxine) (XXII) of von Euler and collaborators (1932) to the complex ergot alkaloids, yohimbine, rauwolfine, calycanthine, etc., which I must be content to merely mention. The fact that strychnine and brucine are indole derivatives was first definitely proved by the examination

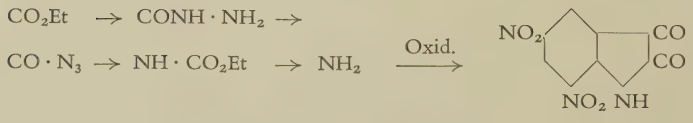


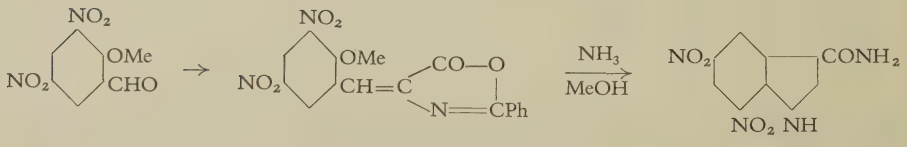
(XXII)

of dinitro-strycholcarboxylic acid (XXIII), which was obtained by Tafel by simultaneous oxidation and nitration of strychnine. It was systematically degraded to dinitro-isatin (with K. N. Menon, 1931), and dinitrostrychol (XXIV) was later synthesized (with P. Hill, 1933).



(XXIII) (XXIV)

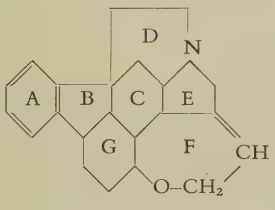




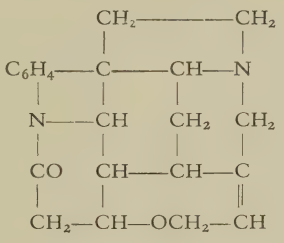
The interlocking developments of the chemistry of strychnine and brucine cannot be usefully summarized and demand separate treatment. This is a most fascinating molecule and with its seven fused rings it is a kind of organic chemist's playground. For many years to come it will provide material for degradative studies. Nevertheless I believe that the problem of its constitu-

tion is finally solved in every detail. For a long time the formula (XXV) seemed satisfactory but in 1945 Prelog and Szpilfogel gave good reasons for supposing that ring E is six-membered. Other evidence in the same direction has since accumulated. The chemistry of neostrychnine, an isomeride depending on a change in position of a double bond, created some temporary difficulties. These have been overcome by the recognition that neostrychnine contains $\overset{\alpha}{N}—CH=\overset{\beta}{C}$, which can be oxidized by perbenzoic acid to $N \cdot CHOCO$, and changed by bromine to a bromo-hydrobromide which in warm water becomes $N—\overset{\beta}{C}—\overset{\alpha}{C}HO$, HBr (with R. N. Chakravarti, 1945–1946). After careful and prolonged consideration I am satisfied that strychnine is (XXVI) (*Experientia*, 1946) and that no alternative can now be entertained.

A tribute must be paid here to the memory of Hermann Leuchs whose brilliant experimental work is admired by all students of this subject.

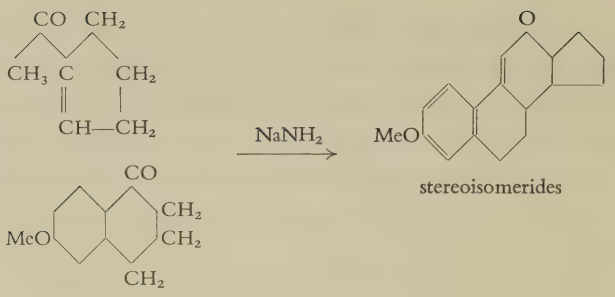


(XXV)



(XXVI)

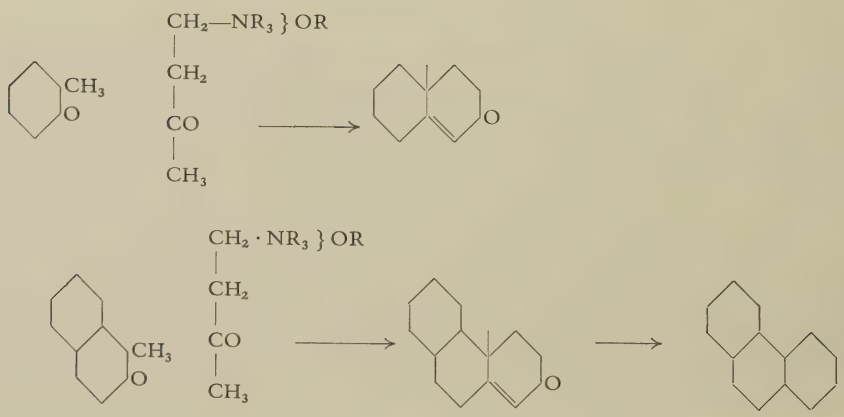
Synthesis in the steroid groups. This investigation sprang from a new point of departure and was at first a survey of the methods applicable to the building of the ring skeletons of the steroid hormones. Again it cannot be shortly summarized and I will speak of a few sections only. The early methods (with E. Schlittler and J. Walker, 1935–1937) encountered stereochemical obstacles when directed towards oestrone; Bachmann made much better use of these routes in his synthesis of equilenin. We always tried to shorten the synthesis by coupling together two components and one promising device (with W. Rapson and D. A. Peak, 1936–1937) is shown in the scheme:

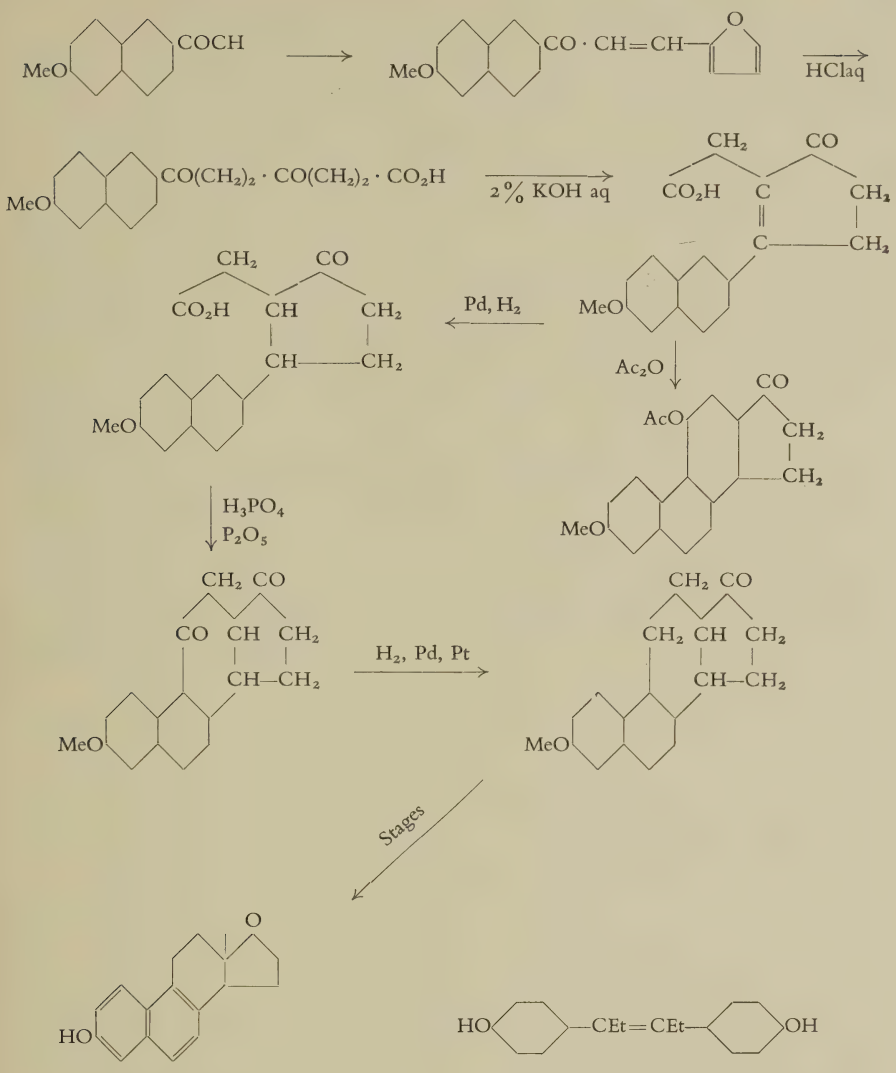


A synthesis of isoequilenin (XXVII) (1938, and with A. Koebner, 1941; also with (Mrs.) R. Jaeger and A. F. Birch, 1945) proceeds by way of some very easily accessible substances, representing the most convenient method of procuring cyclopentenophenanthrene derivatives (see scheme on p. 183).

In the last stages the methylene group adjacent to carbonyl was changed to : C : CH · NMePh, and the angle-methine group was then methylated and the protecting group removed by hydrolysis. Incidently it may be mentioned that co-operation with Professor E. C. Dodds led to the synthesis of a number of diphenylethane and stilbene derivatives which were found to be strongly oestrogenic. Excellent recent work by Dr. P. M. F. Bishop has shown that stilboestrol (XXVIII), one of the first of these substances to be produced, is still the most potent synthetic oestrogen in man, claims for other substances are based on animal experiments only.

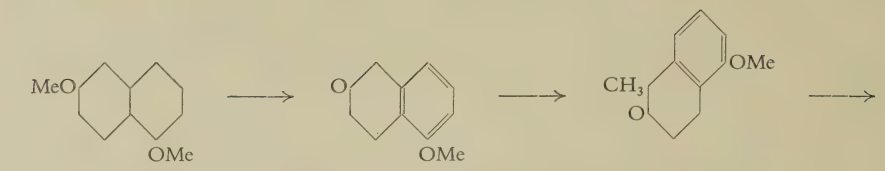
The most hopeful line of synthesis, at present being developed, is that starting with 1:6-dihydroxynaphthalene and employing a method of building on a new ring that was elaborated at an earlier stage. It depends on the use of the metho-salt of a Mannich base as an equivalent of an unsaturated

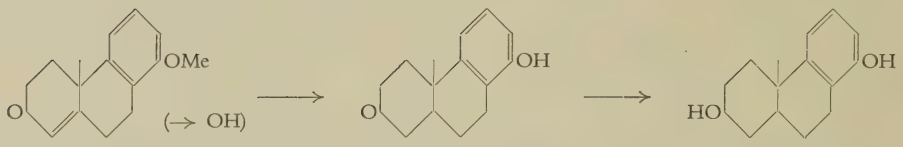


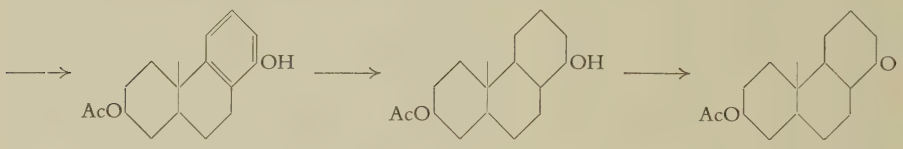


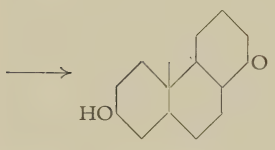
(XXVII) (XXVIII)

ketone. Thus $CH_3CO \cdot CH_2 \cdot CH_2 \cdot NEt_2Me\}$ I, and a basic agent such as $NaNH_2$ or NaOEt, is used instead of $CH_3 \cdot CO \cdot CH{=}CH_2$. Michael-reaction-like addition, followed by ring closure, occurs to cyclic ketones and fortunately attack of —CHMe · CO— seems to be favoured as compared with that of —CH_2 · CO— (with E.C. du Feu and F.J.McQuillin, 1937; with F.Weygand, 1941).



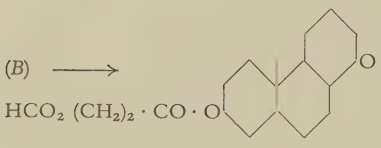






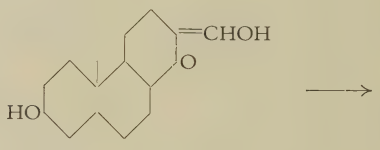
(*stereoisomeride-A: stereoisomeride-B;* 6:1)

(*B*) →



resolved by brucine salt

→ (+) and (—) *B* →



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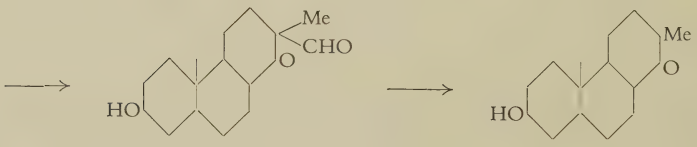

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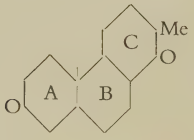


A second precedent investigation is that on the reduction of 2-methoxynaphthalene. With sodium and alcohol this gives a dihydro-derivative, easily hydrolysed by acids to 2-tetralone (with J. W. Cornforth and Mrs. R. H. Cornforth, 1942) (see scheme at bottom of p. 182).

The synthesis shown on p. 184 represents the high-water mark of our achievement in this field and was only made possible by the skill and pertinacity of Dr. J. W. Cornforth.

This last substance from (+) *B* is completely identical with a tricyclic diketone made from a degradation product of desoxycholic acid by H. Reich (1945). The fusion of rings A : B is *cis* and that of B : C is *trans*; hence the prospect for the complete synthesis of coprosterol (and therefore of cholesterol) is a bright one.

I conclude with an expression of deepest gratitude and appreciation to all of my numerous co-workers; any success which I have had was due to their unsparing efforts. Though it might be invidious to mention individuals, yet I may be allowed to say how much I owe to the constant help of my wife, not quite my first, but much my most consistent collaborator, and over the longest period of years.

Biography

Sir Robert Robinson was born at Rufford, near Chesterfield, Derbyshire on September 13th, 1886, the son of William Bradbury Robinson, a surgical dressing manufacturer who invented his own machines for the production of lint, bandages, etc., and the cardboard boxes for packaging them. He was educated at the Chesterfield Grammar School, Fulneck School, near Leeds, and at Manchester University where he graduated B.Sc. in 1906 and D.Sc. in 1910.

In 1912, he was appointed the first Professor of Pure and Applied Organic Chemistry in the University of Sydney. He returned to Britain in 1915 to take the Chair in Organic Chemistry at the University of Liverpool until 1920 when he accepted an appointment as Director of Research at the British Dyestuffs Corporation. One year later, he became Professor of Chemistry at St. Andrews and in 1922 he took the Chair in Organic Chemistry at Manchester University until 1928 when he accepted a similar post in the University of London. In 1930, he was appointed Waynflete Professor of Chemistry, Oxford University, where he remained until his retirement in 1955 when he was appointed Emeritus Professor and Honorary Fellow of Magdalen College. He has been a Director of the Shell Chemical Company and a chemical consultant since 1955.

Sir Robert has been a member of over thirty Government Committees and Chairman of some of them. He was a United Kingdom delegate to the first Conference of UNESCO in 1947. He was knighted in 1939 and appointed to the Order of Merit in 1949.

Robinson's extensive researches in organic chemistry have dealt not only with the structure and synthesis of many organic bodies, but also with the electrochemical mechanism of organic reactions. His interest in the chemical constitution of plant dyestuffs (anthocyanins) soon extended to another group of vegetable bodies, the alkaloids, where the whole series of his researches are remarkable for their brilliant syntheses. He contributed greatly towards the definition of the arrangement of atoms within molecules of morphine, papaverine, narcotine, etc. These discoveries led to the successful

production of certain antimalarial drugs (they are reported in numerous scientific papers, mainly in the Journal of the Chemical Society).

Sir Robert, a Fellow of the Royal Institute of Chemistry and of the Royal Society was President of The Chemical Society, 1939–1941; of the Royal Society, 1945–1950; of the British Association for the Advancement of Science, 1955; and of the Society for the Chemical Industry, 1958. He is a Commandeur de la Légion d'Honneur and holds Honorary Doctorates of over twenty British and foreign universities. He has been honoured by The Chemical Society (Longstaff, Faraday and Flintoff Medals), the Royal Society (Davy, Royal and Copley Medals) and the Swiss, American, French and German Chemical Societies; he has also been awarded the Franklin Medal of the Franklin Institute of Philadelphia, the Albert Gold Medal of the Royal Society of Arts and the Medal of Freedom (U.S. Government). Sir Robert is Corresponding Member, Honorary Fellow, Foreign Member, Associate or Correspondant of almost fifty British and foreign learned societies.

In 1962, The Chemical Society honoured Sir Robert by establishing a Robert Robinson Lectureship, to be delivered biennially *in lieu* of the usual Presidential Address.

In 1912, Sir Robert married Gertrude Maud Walsh, a fellow student at Manchester University. They collaborated in several fields of chemical research, notably in a survey of anthocyanins. She died in 1954; they had one son and one daughter. In 1957, he married Stearn Sylvia Hillstrom (*née* Hershey) of New York.

In his younger days, Sir Robert was a keen mountaineer, having climbed in the Alps, Pyrenees, Norway and New Zealand, and he is an ardent chess player being President of the British Chess Federation, 1950–1953. His hobbies also include photography and music.

Chemistry 1948

ARNE WILHELM KAURIN TISELIUS

«for his research on electrophoresis and adsorption analysis, especially for his discoveries concerning the complex nature of the serum proteins»

Chemistry 1948

Presentation Speech by Professor A. Westgren, Chairman of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Royal Highnesses, Ladies and Gentlemen.

« Scheele analyses the universe on the hearth », it was thus that Tegnér on the occasion of the fiftieth anniversary of the Swedish Academy, described the most dazzling of the works of chemistry in our country in the 18th century. The works of Scheele could not be better summed up in a few words, for analysis, the separation of the components of a body, was the prime essential for him as it was for so many other chemists.

The art of separating the constituents of compounds, chemical analysis, has since been developed almost to the point of perfection. However, for a long time past, difficulties have been encountered in one particular field. The efforts of the research workers have not, in many cases, been able to separate substances composed of large molecules without changing the nature of these molecules in the course of the experiments. This failure was particularly keenly felt because these substances are of prime importance from the point of view of biology and of technology, and it is consequently on them, in our age, that the attention of research workers has been concentrated. Among these substances are the proteins and the carbohydrate polymers which play a predominant part in the vital processes; the first in animal life, the second in plant life. Also among them are the substances composing modern synthetic materials, including the different kinds of synthetic rubber, isolating substances, plastics, pressed material, new textiles, and other products, the practical importance of which is increasing day by day.

At the Institute of Physical Chemistry of Uppsala, research work has long been directed towards the study of the properties of colloids and similar substances with large molecules. It was there that Th. Svedberg perfected his celebrated ultracentrifuge method, which makes it possible to determine the molecular weights of these substances. Finally, it was also there that the problem of the separation and the purification of substances with large molecules was resolved.

Two phenomena were made use of: electrophoresis and adsorption.

In a liquid, particles in suspension or large molecules carry, as a general

rule, electrical charges which differ according to the composition of the surrounding medium. These particles and molecules are consequently displaced if they are subjected to the influence of an electric field, that is to say, if an electric current is made to pass through the solution. This phenomenon is called electrophoresis. The rate of displacement depends not only on the potential difference and the charge of the particles, but also of course, on their size and shape. In this way, one has therefore a possibility to separate one from the other the different kinds of particles or molecules in solution, and one can, if one so wishes, purify them.

Many research workers have worked at this problem for a long time, although on the whole with little success. Electrophoresis is a complicated phenomenon: to use it as a basis for a general analytical method presented such difficulties that all hope of overcoming them had often been given up. It is to Arne Tiselius that we owe success. He began by thoroughly studying electrophoresis and the numerous disturbances which accompany it, and, on the basis of his study, he worked out a rational method. His first results were published in his doctor's thesis in 1930. He has since then, more than once improved his experimental equipment and has in the end brought his method to a high level of perfection.

In this way, it has been possible to obtain exact determinations with regard to the electrophoresis of proteins and as a result these substances can be characterized with more certainty than heretofore. Thanks to the acquisition of an important basis of operations, the difficult chemistry of the proteins will be able to continue to develop.

Tiselius has made many discoveries of far-reaching effect by applying his method of electrophoresis; that globulin, a protein of blood serum, was not an entirely homogeneous substance, had already been supposed: Tiselius succeeded in separating this seroglobulin into three distinct parts, each comprising slightly different groups of molecules. This finding is at the root of research work of the utmost importance for practical medicine, which was carried out in the United States during the last World War – research work aimed at dividing human blood plasma into fractional parts. If American scientists had not had at their disposal Tiselius' method as a control, they would probably have failed when they tried to solve that problem. In the course of their research work on electrophoresis, Tiselius and his collaborators also carried out experiments of great medical value on the antibodies of a protein nature which are formed in the blood during immunization.

With the electrophoresis method, it is possible to study only colloids and substances with giant molecules. But it is equally necessary to possess a sensitive method of analysis for substances of slightly smaller molecular weight – one might say, of medium molecular weight; to this end, therefore, Tiselius perfected analysis by adsorption. This method has been known in principle for quite a long time. A little more than forty years ago, the Russian botanist Tsvett derived from it a process which is of practical use for separating plant pigments. In this first, somewhat primitive, form, the method can really only be said to apply to coloured substances and is therefore called chromatographic analysis.

Analysis by adsorption is founded on the phenomenon which also serves as a basis for the design of contemporary gas-masks. These, as you know, comprise a filter cartridge of powdered carbon which keeps back toxic products when air is inhaled. In the same way, a solution which is made to pass through a powdery, compressed mass or through any porous mass, leaves behind it some of the substances in solution: these settle more or less on the surface of the solid medium, a phenomenon which is called adsorption. If there are several substances in solution at the same time, they are more often than not adsorbed in different ratios and this fact can be used to separate them. Tiselius, after systematic research work bearing on the conditions of analysis by adsorption, has established methods of operation differing in their principle. In these processes, the methods of observation are such that it does not matter whether one analyses coloured or colourless substances. It was also a decisive step forward when Tiselius discovered the means which allow analysis by adsorption to be used not only qualitatively but quantitatively. His research work in that field was fertile in new ideas and was conducted with great ingenuity. It is not yet finished but on the contrary is now entering a very promising phase. The method of analysis by adsorption will certainly be perfected in the future thanks to the efforts of Tiselius and his fellow workers. It has already developed to the extent of having become a research process of immense value.

Fine results have been obtained by Tiselius for the analysis of mixtures comprising amino acids, peptides, and sugars. His collaborator, Stig Claesson who, during these past years, has made a valuable contribution to the working-out of the method, has applied it with success to other groups of organic substances.

The value of the new methods which have been briefly described here, is especially brought to light by their use, which is nowadays general, in

international research in biochemistry and in medicine. Tiselius' apparatuses for electrophoresis and analysis by adsorption nowadays form part of the normal equipment of a great number of laboratories and medical institutes not only in Sweden but also abroad. One notices continually in chemical periodicals new experiments made by using Tiselius' methods.

Therefore it is with the full conviction of acting in accordance with the opinion of international chemical circles that the Academy of Sciences has decided to award to Arne Tiselius this year's Nobel Prize for Chemistry on the grounds of his research work on electrophoresis and on analysis by adsorption and, in particular, for his discoveries concerning the heterogeneous nature of the proteins of the serum.

Professor Tiselius. In contrast to many present-day scientists, who are specialists, often each in a very narrow sphere, you possess deep knowledge in several fields. Your very great competence extends not only to physical chemistry and to biochemistry, but equally covers large stretches of physics and medicine. It would be quite natural to ask oneself if, in an age of scientific organization conscious of goals to attain, a group of scientists made up of representatives from those different disciplines had been given the task of making a concerted attack on the problems which you have solved, they would have succeeded as well as you have done. I do not think it very likely. The words uttered last year by Eric Linklater in a speech to the students of Aberdeen University contain an irrefutable truth: « The individual mind may, in certain activities, be more competent than the conjoint mind of a committee, and the imagination that lives in a man alone has no home in a board of directors... Hamlet was not written by a convocation of literary critics. »

It is with a justifiable pride and sincere joy that the Academy of Sciences has had this year the opportunity of selecting as Nobel Prize winner a member of its own society. Your work has been for the Academy a source of joy, all the greater for the fact that numerous indications show that your scientific career is continuing its upward course. The Academy charges me to offer you its deep respect and its enthusiastic good wishes. And now I ask you to receive the prize from the hands of His Royal Highness, the Crown Prince.

Arne W. K. Tiselius

Electrophoresis and adsorption analysis as aids in investigations of large molecular weight substances and their breakdown products

Nobel Lecture, December 13, 1948

The methods, whose development and use I shall attempt to describe in this lecture, have a common characteristic: they are based on certain specific physico-chemical properties of the substances to be studied, namely, their migration in an electric field (*electrophoresis*) or their affinity to the surface of a finely divided solid substance (*adsorption*). These phenomena are specific in the sense that they often display wide variation from substance to substance and therefore are well adapted for the characterization of certain substances which are otherwise difficult to define like proteins and other biocolloids. Electrophoresis and especially adsorption would not, however, have come to play their present roll in chemistry if they had only been utilized for the characterization of different substances. Fundamentally, the significance of these methods up to the present time lies in the opportunities which they offer for making use of these phenomena for a rational *separation* of substances in solution under conditions which are especially favourable to many substances of biochemical significance.

Separation methods for preparative and analytical purposes are, as is well known, of fundamental importance in the chemists' work. It is illuminating that in the Dutch language « Scheikunde » or the Art of Separation, is even today often used as a name for chemistry. Almost daily the biochemist is faced with separation problems in his laboratory. Such is the case when he works with substances belonging to the common organic substances of more or less known structure, and is true to an even greater extent if he is interested in complex substances of large molecular weight, for example: proteins, enzymes, viruses, polysaccharides, and nucleic acids, whose detailed structure is as yet unknown, but which on account of their great significance in fundamental life processes are objects of especially intensive study at present. In these branches of bio-chemistry some of the most important problems are to *isolate* the substances which are responsible for a specific biological or biochemical effect (e.g. certain enzymes) and also to *define*

and *characterize* these substances as accurately as possible. I particularly wish to emphasize that the definition of a chemical entity, which just in this particular field is often extremely difficult, ultimately depends on the separation methods we have at our disposal, or those which are thought to be suitable for use without danger of altering the substance in question. Thus, the problem of separation is of the utmost interest far beyond preparative aspects, and can often supply important information about the existence of biochemically significant complexes, for example between a prosthetic group and a protein in an enzyme. But, in addition to this, fundamental problems in the chemistry of large molecules are to a high degree dependent on the development of suitable and highly specific separation methods. Each attempt to elucidate the chemical structure of these complex substances depends appreciably on the possibility of studying the structure of simpler fragments, which are obtained by breaking down the molecules in some way. The complicated mixtures thus obtained are, however, extremely difficult to separate with the methods now available, particularly if an attempt is made to isolate products resulting from a partial breakdown.

With these words I have tried to give an idea of why we have felt it worth-while to devote a great deal of work to the problem of separation within this field of chemistry. I will principally confine the following discussion to a consideration of the two methods to which the major portion of our work has been devoted.

Unfortunately, time will not allow me to discuss a number of other methods with similar uses, despite the fact that just recently many important advances have been made in this respect.

In principle, two different possibilities may be made use of in order to follow the migration of dissolved substances in an electric field: the *transference* and *moving boundary* methods*. Both have been known for a long time. As early as 1853, Hittorf studied the transference of the constituent parts of inorganic salts, acids, and bases with an electric current, and in 1886 Oliver Lodge made use of the moving boundary method. Comprehensive studies of the «cataphoresis» or «electrophoresis» – as the phenomenon was called – of proteins and enzymes were undertaken at the beginning of the century by Hardy in England and Michaelis in Germany. My own work on the electrophoresis of proteins commenced in 1925 as a continuation of

* A third possibility: the direct observation of the migration by means of microscope or ultramicroscope has found important use in colloidal chemistry but is of course limited to cases where the particles are sufficiently large to be observed in this manner.

some experiments by Svedberg, Jette and Scott, in which the moving boundaries of colourless proteins were made visible by fluorescent photography. As this method suffers from several disadvantages I attempted instead to use the method of observation introduced by Svedberg for the observation of ultracentrifugation, namely, photographing in the short-wave ultraviolet light with the use of quartz lenses and with a light filter containing chlorine and bromine. This work was later extended to include several proteins as well as mixtures of proteins. A great advantage of the boundary methods is that the migration of the different components in a mixture can be followed. With the transference method this is not possible unless some convenient method is available for the determination of the amount of each component individually in the limbs of the electrophoresis tube. The experiments which were performed on mixtures and on non-homogeneous proteins up to 1930 with the light-adsorption method clearly showed the usefulness of the electrophoretic method for «electrophoretic analysis». But it also became obvious that a number of technical difficulties would have to be overcome in order that the method should have the resolution capacity required for it to be of value as a general useful tool in biochemistry.

On the basis of experimental and theoretical studies on the numerous sources of error, I undertook (1936–1937) a radical reconstruction of the electrophoresis apparatus. With the aid of some pictures I would now like to discuss the most important technical improvements which distinguish the

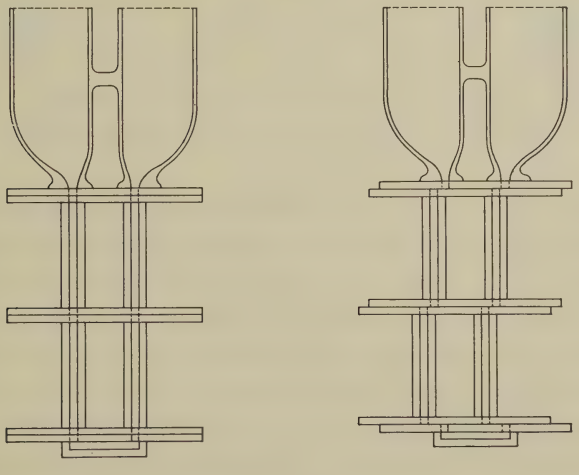


Fig. 1. Electrophoresis U-tube composed of rectangular glass cuvettes.

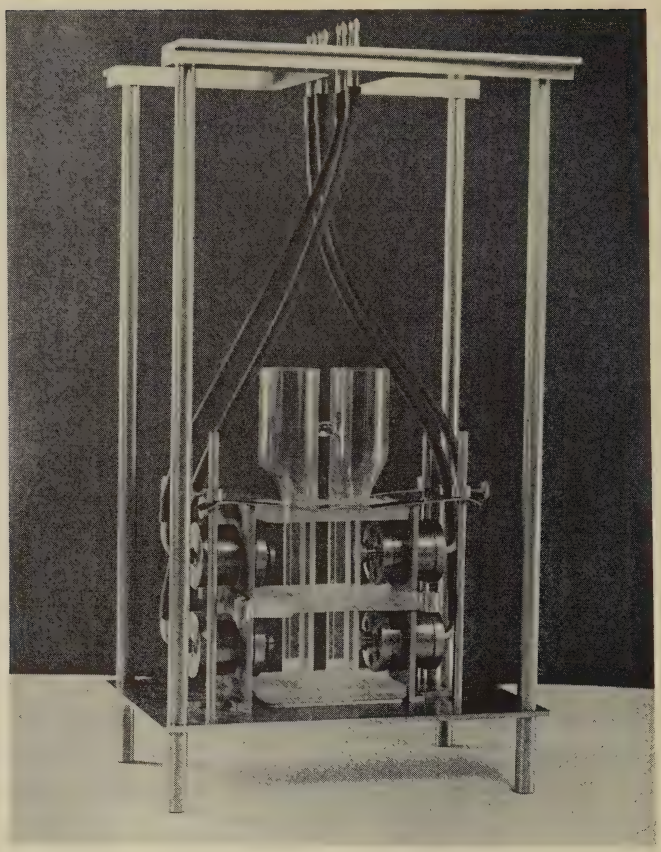


Fig. 2. Electrophoresis U-tube mounted in its stand with pneumatic arrangements for shifting the cells.

new apparatus, and at the same time to mention a number of additional improvements which were later made in Uppsala, principally by Harry Svensson.

The greatest difficulty which is encountered in electrophoretic analysis, when one wishes to increase the resolution capacity by increasing the voltage on the apparatus is due to the fact that the heat of current causes density differences in the solution in the electrophoresis tube. When these reach a value of the same order of magnitude as the density differences across the boundaries the latter are destroyed by convection currents. Even at a somewhat lower load the diffuse parts of the boundaries are distorted and it is impossible to complete the experiment. These distortions can be studied independent of other effects if one uses alternating instead of direct current. There is,

however, a simple way to substantially reduce these distortions. Water, as is well known, has a density maximum at + 4°C. By performing the experiment in a cold thermostat in the neighbourhood of + 4°C the effect caused by the unequal heating is minimized as the density differences for the most part are substantially reduced. Experiments show that by this simple arrangement the load on the apparatus can be increased to an extent corresponding to a potential gradient 8–10 times as high as at room temperature. This is of especial value for experiments with those proteins, which require the presence of considerable amounts of salts in order to stay in solution, as for example, the globulins in blood serum.

Also the form of the electrophoresis tube is of great importance in view of the fact that the convection currents must be reduced to the greatest

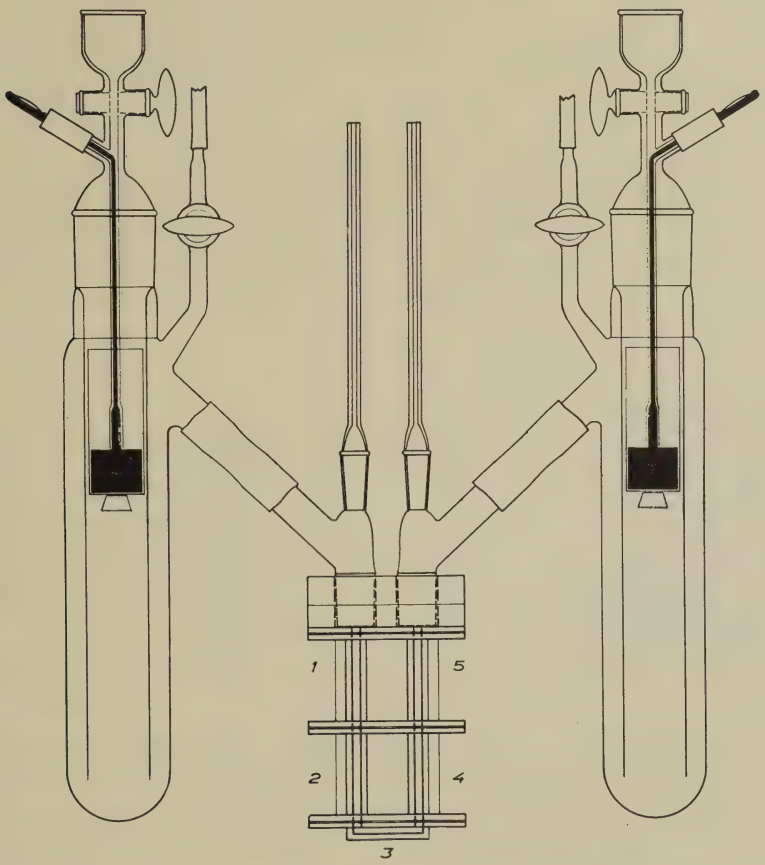


Fig. 3. Electrophoresis U-tube assembled with electrode containers for reversible electrodes.

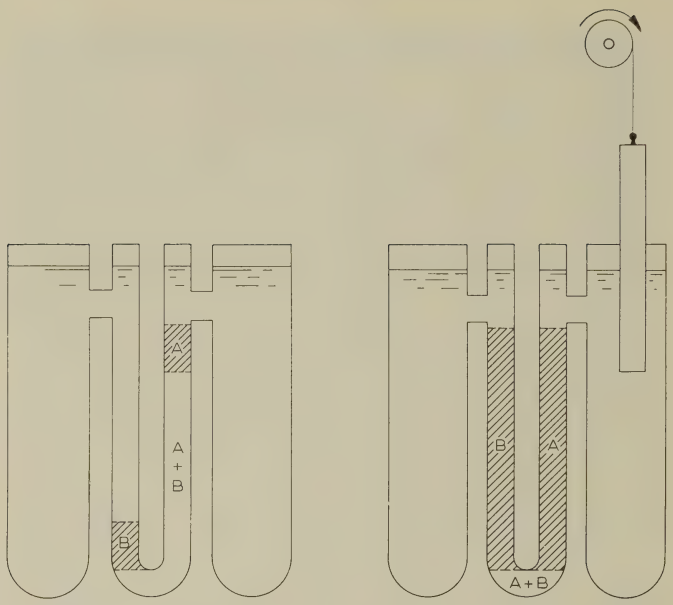


Fig. 4. Compensation arrangement. A slow depression of the plunger into one electrode vessel (*to the right in the figure*), by clockworks, causes a slow and accurately known shift of the solution in the U-tube in a direction opposed to that of the migration. By this means the separation of two substances A and B can be carried out much farther than without compensation (*compare with picture to the left*).

possible extent. Instead of a cylindrical tube, a tube was introduced with a rectangular cross section. The final model, which is shown in Figs. 1–3 was designed in order to facilitate the application of optical methods of observation and in order to provide a convenient way of forming boundaries at the beginning of the experiment as well as to facilitate the removal of samples at its termination. The tube is composed of sections made of acid-fast cemented plane-glass plates. These sections lie against one another with horizontal ground glass plates between, and can be moved in relation to each other in the horizontal direction with a pneumatic arrangement (Fig. 2).

Using this construction it is possible to perform the electrophoretic analysis during one experiment with both boundary and transference methods. The latter method's applicability in enzyme chemistry had already been demonstrated by Michaelis, but an electrophoretic analysis of enzymes was first performed by Hugo Theorell in the middle thirties with a modified transference apparatus of his own construction. This work which among other things led to the clarifaction of the nature of Warburg's yellow en-

zyme, contributed in a large extent to making the electrophoretic method known.

Additional technical details of significance in the new construction have been the electrode vessels of ample size with their reversible Ag-AgCl electrodes together with the so-called compensation or the counter-current device. The latter has made it possible to make maximal use of the electrophoresis tube's capacity even in the case when fast components would migrate out of the tube before an appreciable separation has taken place.* The improved type used most frequently nowadays is of somewhat different construction which makes use of an injection syringe operated by an adjustable clockwork or a synchronous motor.

The optical method used for the observation of the boundaries is based on the localization and quantitative determination of refractive index gradients due to the boundaries in the electrophoresis tube.

Such a method is considerably more sensitive than those based upon light absorption when the detailed concentration distribution in the electrophoresis tube is to be studied. Before the new apparatus was constructed, I had tried observation methods based upon the curvature of a light beam in a concentration gradient, and for the first form of the new method, the simplest of these was chosen, namely the Toepler «schlieren» method. Some typical «schlieren» photographs of electrophoresis of a uniform protein and of mixtures are shown in Fig. 5. The method is well suited for the localiza-

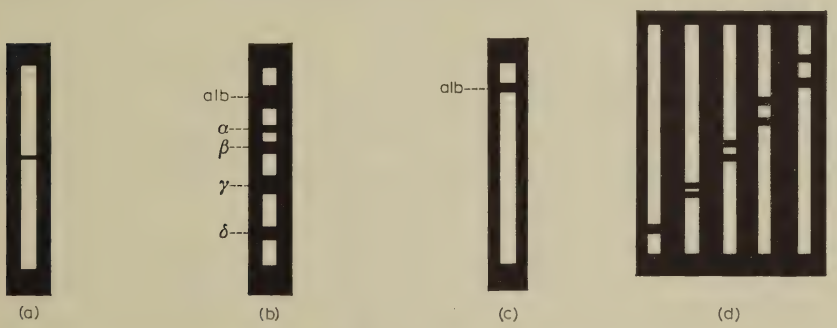


Fig. 5. Schlieren photographs of an electrophoresis cell showing migrating boundaries. (*a*) Egg albumin; (*b*) horse serum; (*c*) serum albumin, isolated from horse serum by electrophoresis; (*d*) a mixture of egg albumins from guinea hen and duck (according to Landsteiner and Van der Scheer).

* The compensator's mode of operation is shown schematically in Fig. 4 and should speak for itself.

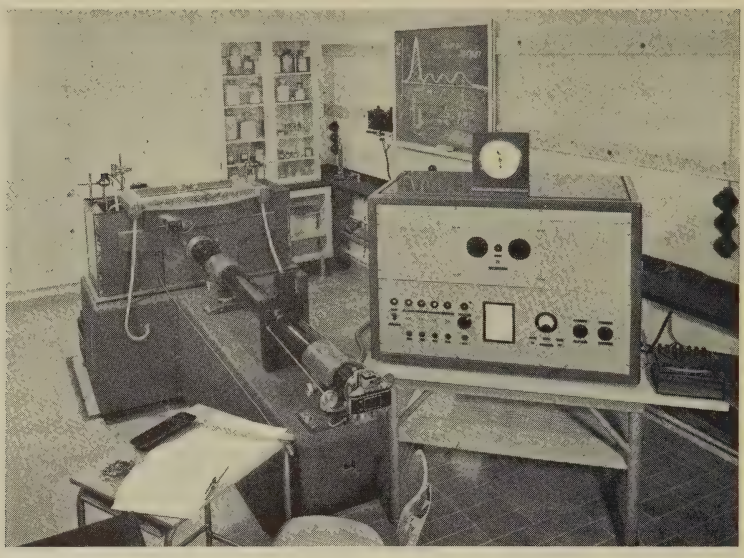


Fig. 6. A complete electrophoresis apparatus with schlieren optics according to Svensson.

tion of the boundaries, but must be developed further if one wants a complete record of the concentration distribution in one and the same exposure. This can be arranged in several different ways: a mechanical coupling between the plate-holder and the schlieren diagram (Longsworth), application of a schlieren slit and a cylindric lens (Philpot, Svensson). Fig. 6 shows a complete arrangement for electrophoresis with optical equipment according to Svensson.

I will show some figures which illustrate the type of electrophoresis diagram obtained by these observation methods, and which at the same time may serve as examples of the use of electrophoretic analysis in the study of proteins. Fig. 7 shows some electrophoresis diagrams from experiments with

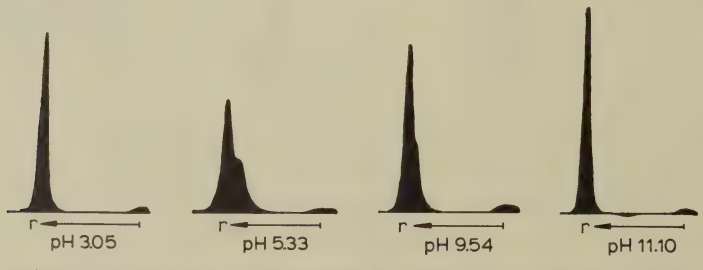


Fig. 7. An electrophoresis diagram taken by Longsworth's scanning method, showing the inhomogeneity of egg albumin at certain pH's (according to Longsworth).

egg albumin, which in general is considered to be a relatively well-defined protein. At certain pH values it shows complex diagram, as seen in the figure, indicating inhomogeneity. In a very large number of investigations of proteins, enzymes, antibodies, viruses, etc. the method has been used as a criterion for purity in this manner, and has often shown that the substances are more complex than was originally supposed. On the other hand one must not, from an apparently uniform diagram containing a single peak, draw the conclusion that the material is homogeneous. Electrophoresis is only one of the many criteria which must be used to obtain decisive information in this respect.

As an aid and guide in preparative work, electrophoresis is often of great value. Step by step it is possible to follow the preparation and see how one component partially or completely separates from another just in the manner spectral analysis is made use of in inorganic chemistry.

The method has been of importance in the problem of fractionating blood serum. Among the first results obtained by the new apparatus was the fact, that in electrophoresis, serum gives a number of relatively distinct components, namely: albumin, α, β, and γ globulin (see diagram Fig. 5 b and Fig. 8). It was subsequently found that further subdivision of some of these components could be made. During the course of extensive investigations on serum fractionation, which were performed in the U.S.A. during the recent war, and particularly by Edwin Cohn and his co-workers, electrophoresis and ultracentrifugation were used as controls during the fractionation procedure. Fig. 9 shows a number of electrophoresis diagrams obtained from such a fractionation.

Some other applications in protein chemistry ought to be mentioned. Horsfall and I have shown, through electrophoretic analyses, that the reversible dissociation of hemocyanins is conditioned by factors possessing pronounced biochemical specificity, so that closely related species can form mixed molecules by the reassociation of the dissociation products, which is not possible with hemocyanins of more distant species.

The electrophoresis diagram also gives the migration velocities of the different components at the particular pH and salt medium in which the experiment is being conducted. It is also possible to obtain a quantitative estimation of the relative amounts of components present, at least under certain conditions. A knowledge of these conditions is a necessary prerequisite for the successful use of electrophoretic analysis even when apparatus construction is quite perfect. The ideal case for electrophoretic migration is a con-

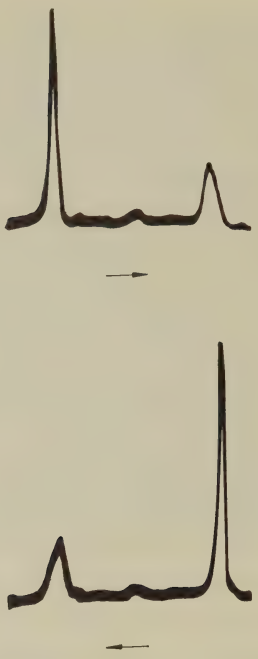


Fig. 8. Electrophoresis diagram by Svensson's optical method of a pathological serum from a patient with an abnormally high percentage of γ-globulin.

stant potential gradient and with constant conductivity throughout the entire electrophoresis tube. These conditions are however never completely fulfilled, as the migrating substances themselves have a certain conductivity, and therefore eventual differences in the potential gradient can be further accentuated through the accumulation or spreading out of the buffer ions migrating in the inhomogeneous field. The theory for these effects and the manner in which they influence the boundaries' migration has been worked out by Kohlrausch, Weber, and Von Laue, and further developed with special regard to electrophoretic analysis by Svensson, Dole, and several others. One would expect these phenomena, which we have called boundary anomalies, to be present, when the substances under investigation are responsible for an important part of the total electrical transport through the electrophoresis tube. From this it follows that experiments should be performed in media of high conductivity and that even then only substances of comparatively low specific conductivity are suitable for electrophoresis, for example, proteins and many other high molecular weight substances. A lack of symmetry in the observations of the two limbs of the electrophoresis

tube with regard to mobility, the number of boundaries, or the relative amounts observed for the different components is a sign that such anomalies interfere. In most electrophoretic measurements these anomalies are not sufficiently eliminated. However, it is possible with the aid of the above-mentioned theoretical analysis to predict to a certain extent the most favourable conditions for eliminating the sources of error. Moreover, it is possible from two or more experiments, by utilizing different salt or protein concentrations to extrapolate to the value which should be obtained at zero protein concentration or at a high salt content.

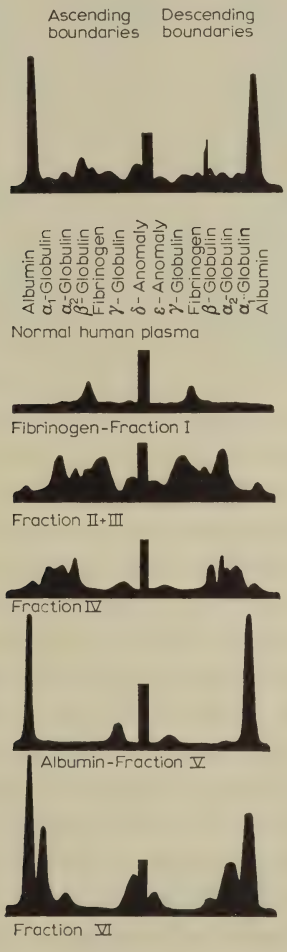


Fig. 9. Electrophoresis diagrams from different stages in the fractionation of human plasma (according to Cohn).

It is apparent from the above discussion that further improvements in the apparatus and method must above all be aimed at the possibility of performing the experiment with as low concentrations of the substances studied as is possible. This implies further increasing the sensitivity of the optical method, a problem which should be quite possible to solve. At present work in this direction is in progress at Uppsala as well as elsewhere. By such improvements one ought to gain not only an increased accuracy in electrophoretic analysis in general, but also an extension of the applicability of the method to substances of lower molecular weight, for example peptides.

The advantages of the electrophoretic method are above all its gentleness and its marked specificity. During the entire course of a separation the substance remains in solution of approximately constant composition, thereby eliminating the risk of denaturation and other irreversible changes which so easily influence processes dependent on precipitation. This advantage is also characteristic of separation by ultracentrifugation. Frequently proteins, enzymes, and other substances which have been purified by precipitation or by repeated recrystallization are nevertheless found by electrophoretic analysis to be inhomogeneous. This is probably due to the fact that substances of such a nature show a great tendency to carry foreign substances with them on precipitation. The components are more likely to be free from one another in solution, and as the electrophoretic separation depends only on differences in mobilities it is of little importance if the impurities are present only in very small quantities. The separation proceeds just about as readily – in contrast to what is usually the case in most other separation methods.

The method's great convenience has, however, certain limitations which should be indicated. In electrophoretic migration only those molecules or particles which are free from one another in the solution are separated. If complex groups or associations of some type exist, a separation can only be expected to the extent in which these complexes are dissociated. A component which under certain conditions in electrophoresis is observed to be homogeneous can also very well, under different conditions (with less gentle treatment or merely by a change of pH) show itself to be of complex nature. However, electrophoretic (as ultracentrifugal) observations are of considerable interest also in such a case from an entirely different point of view. The gentleness of the method should often make it possible to draw the conclusion that such demonstrated complexes may even be present in the original material, that is to say, when we deal with proteins, in the living organism itself. It is not necessary to be a vitalist in order to express the

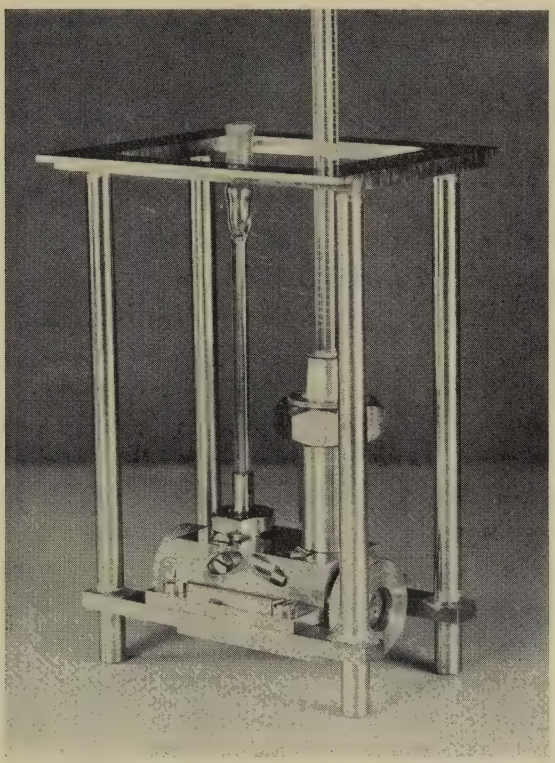


Fig. 10. Micro-interferometer for adsorption analysis. The capillaries of the interferometer lie horizontally in a brass block. The solution is allowed to run from a burette through a filter containing the adsorbent (*to the right*). The outlet tube is seen to the left.

opinion, that what from a chemist's viewpoint may be taken as a strongly retained impurity (for example the lipid in a lipo-protein complex), from a biological viewpoint perhaps may possess a vital function. Throughout the course of biochemistry – quite naturally – principal interest has been directed to the chemical entities that have been isolated from biological materials, but there is little doubt that in the future much closer attention must be paid also to the existence of specific complexes in the living organism. It is the chain of events with linked reactions which is the key note of life's processes and this interrelationship or linkage can perhaps best be conceived as the result of an association of substances with different specific functions.

The problems of separation and characterization of high molecular substances, which I spoke of at the beginning of this lecture as a leading theme for our work, cannot be solved by only one method – in this field one must

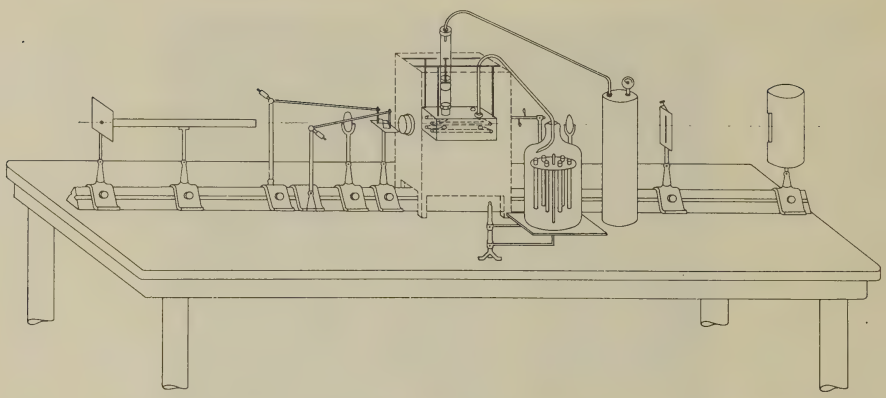


Fig. 11. Schematic diagram of an apparatus for interferometric adsorption analysis.

be especially prepared to apply different methods of procedure before drawing definite conclusions. The multitude of substances which must be dealt with, even from a single kind of original material, sets a very high requirement for the specificity of the methods. Electrophoretic specificity is often pronounced – I may refer to the picture of the separation of different egg albumins (Fig. 5 d). But in other cases it is quite insufficient. As an example I may point to the fact that the method does not allow for differentiation between normal and immune γ globulin in immune sera. It is of course natural that a method which only makes use of the differences in electrochemical qualities cannot fulfil the requirements in all conceivable cases.

In 1940 I began some experiments in order to make use of the chromatographic analysis in the separation of proteins and of their breakdown products. This was considered worth a trial on account of the remarkable specificity which this separation method shows for different organic substances, which had become evident in the course of numerous investigations during the years following the original work of Tsvett in 1906. The particular difficulties which had been encountered (such as boundary anomalies) in the electrophoresis of peptides and similar breakdown products of proteins made it also desirable to find a method especially adaptable for the separation of these substances.

The method which was subsequently worked out was intended to attain a high degree of precision for the observation of even very small separations and was from the beginning developed for work on colourless substances, for which the ordinary method of observing the migration of the zones directly on the column cannot be used.

In principle the method involves the continuous measurement of a solution's concentration by some convenient method – after the solution has passed through a column of adsorbent. Usually the measurements are made with a micro-interferometer. The adsorbent is packed into cylindrical cells made of gold-plated brass or of perspex which are then coupled to the interferometer (Fig. 10). After emerging from the interferometer, the solution runs out into graduated test tubes whose position can be changed as the different fractions emerge. Fig. 11 shows the arrangement, with the interferometer in a thermostat; the optical parts, together with a compressed-air tank, used to provide the necessary pressure for the filtration through the column. In working out the method Dr. Claesson has made very valuable contributions, particularly by his construction of the micro-interferometric apparatus just described.

The concentration as expressed in terms of the refractive index is plotted

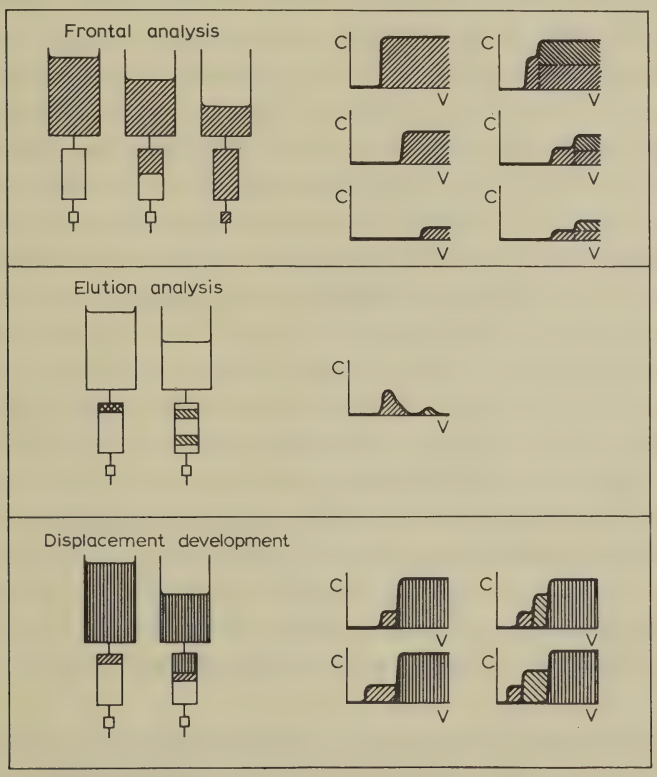


Fig. 12. Different types of adsorption analysis.

in a diagram as a function of the volume which has passed through the filter. Schematic diagrams of this type are shown in Fig. 12. The three curves represent the methods, somewhat different in principle, which are used for this type of adsorption analysis. In the first case the solution to be investigated is allowed to run through the column uninterruptedly and the curve furnishes the form of the adsorption front with a differential separation of the same type as that accomplished by electrophoresis. We have called this *frontal analysis*. Each new step represents a component, and the height of the step is proportional to the component's concentration in the original solution when the different components are adsorbed independently of one another. However, this is the case only with very dilute solutions or in cases of very weak adsorption. Claesson, who has devoted himself especially to this type of adsorption analysis, has shown, that the law which governs adsorption in homologous series (Traube's rule) makes it possible to apply a relatively simple correction for the effect of mutual displacement. He has used this method, among others, for the analysis of mixtures of the higher fatty acids. The second method, *elution*, only requires a smaller quantity of the material, which is adsorbed on the top of the column after which a pure solvent is pressed through the column. The components emerge as completely separated peaks in the diagram. We have not used this method a great deal because, at least in cases of stronger adsorption, the peaks are too much spread out and the concentration becomes too low for our interferometer. The third curve shows the so-called *displacement analysis*. Here also a small quantity is first pressed into the column, after which a solution of a suitable displacing agent is pressed through – that is to say, a substance which is adsorbed more strongly than any of the components present in the test solution. It may easily be shown that when the displacing agent's front passes down through the adsorption column, there develops before the front a system of closely adjoining zones, each one containing a pure component. The concentration of each zone depends only on the coefficient of adsorption, while the zonal width is proportional to the quantity of the component in question. Thus a complete separation is effected without the admixture of an elution agent and the resultant diagram makes it possible to identify the components and estimate the relative quantities of each.

In principle it is of course possible to use any convenient method – physical or chemical – in order to follow the processes of adsorption in this manner. It is also possible, by means of a suitable automatic sampler to collect

successive fractions of the solution emerging from the column in order to make subsequent determinations of the percentage of dissolved substance in each fraction. The latter method or procedure has been used particularly by Moore and Stein at the Rockefeller Institute in New York, and has been found most convenient for experiments with low concentrations of material or for experiments which take a long time to run – it is certainly not convenient to make continual interferometric observations over a period of days. On the other hand continuous observation is necessary when frontal analyses are being performed or where there are many components with only slightly different adsorption.

Just as chromatographic methods in general, these methods are useful for the analysis of substances of the most varied sort. We have performed a very large number of model experiments on amino acids, peptides, sugars, fatty acids and fats, etc. As an illustration I will show some pictures which demonstrate some applications of the procedure. Fig. 13 shows the results of a frontal analysis conducted on a mixture of homologous fatty acids (Claesson). Fig. 14 shows a displacement analysis with carbohydrates, and Fig. 15 a displacement analysis of peptides. (For further details see the figure captions.)

The principal interest in adsorption analysis of the type described lies however in the possibilities for its use on large or intermediate-sized molecules – if the latter phrase is understood to mean the larger breakdown products of high molecular substances. For many of these substances, amino acids, lower peptides and different sugars, simple and effective chromatographic

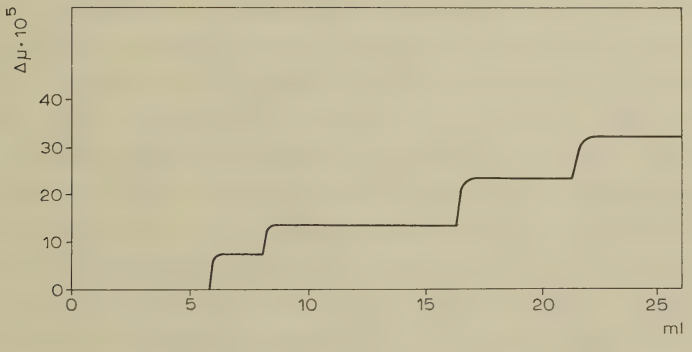


Fig. 13. Frontal analysis of a mixture of four fatty acids, with 8, 10, 14, and 16 carbon atoms in ethyl alcohol, with active carbon as an adsorbent. *Abscissa*: volume. *Ordinate*: refractive-index increment (Claesson).

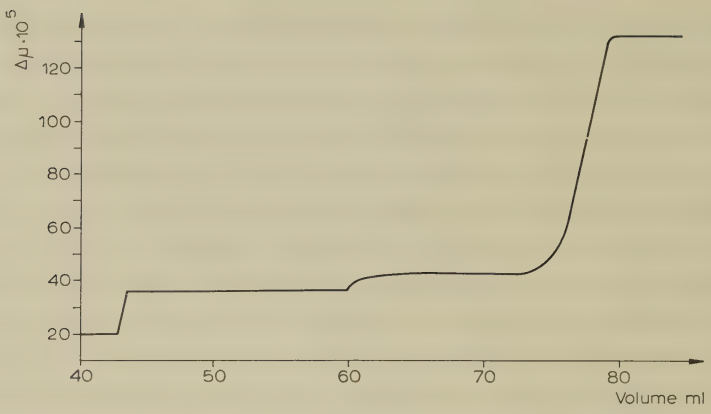


Fig. 14. Displacement analysis on carbon of a mixture of 25 mg sucrose and 25 mg maltose. (Displacing agent: 0.5% phenol.)

methods are now available, particularly the excellent methods for partition chromatography worked out by Martin and Synge. Thus there is hardly any reason for using the more complicated methods described above on such substances except in special cases. Of course the larger the molecules one has to deal with, the more difficult it becomes in general to find a suitable pair of solvents for use in partition experiments. In such cases it may generally be easier to find suitable adsorbents and to make use of these in frontal or displacement analyses in the manner I have just described.

These methods are still in the process of development, and I will only mention a few examples of their use in the work now in progress. Fig. 16 shows a frontal analysis of insulin which was partially broken down by oxidation, and the solution may be interpreted as containing at least four components. Fig. 17 has been taken from a recently published work by Claesson on the adsorption analysis of high polymers and shows the composition (weight frequency) as a function of the retention volume for three different polymethylmetacrylates, as obtained by frontal analysis.

For a quantitative estimation of these diagrams it is of the utmost importance that as low a concentration as possible can be used. It is accordingly of great interest that Claesson has recently been successful in constructing a new adsorption analysis interferometer with approximately five times the sensitivity of the previous model. The large separation of high polymers of the polymetacrylate and nitrocellulose types which Claesson has succeeded in accomplishing indicates that adsorption analysis in this field will come to assume great importance.

For proteins and similar substances the immediate problem is to find suitable adsorbents. Above all, a relatively quick and reversible adsorption process is required for chromatographic analyses, and for this reason many adsorbents commonly used in biochemistry cannot be used for these substances. The advantages in the use of the chromatographic procedure – in the form just described or with some similar method – are however so great that it appears worth-while to search for such adsorbents. The advantage of using an adsorption column in comparison to the simple one-stage operation (« batch process ») is almost comparable to the benefits accrued from the use of a distillation column instead of a simple evaporation procedure for the fractionation of fluid mixtures. Just recently we have studied an adsorption phenomenon previously hardly known, which we have called *salting out adsorption*, which possibly may fulfil the recently mentioned requirement

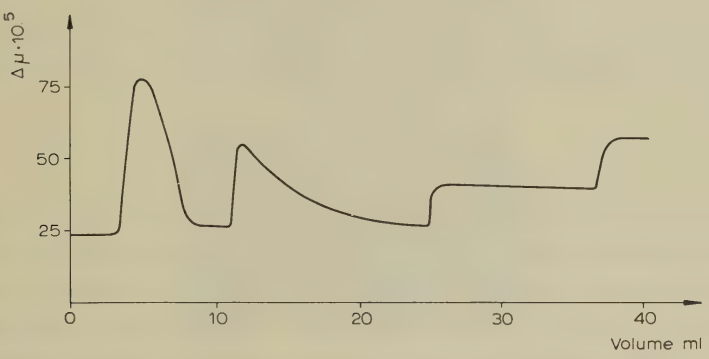


Fig. 15. Displacement analysis of valine, leucine, methionine. (Displacing agent: 0.5% ethylacetate.)

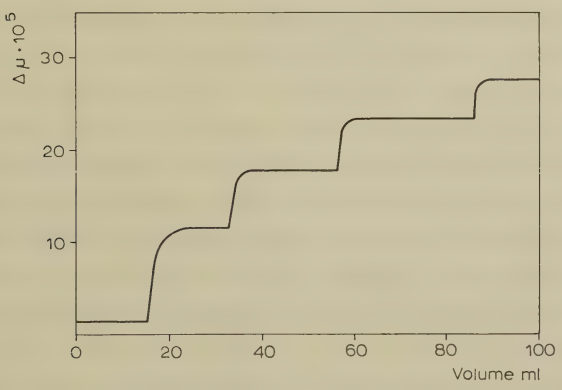


Fig. 16. Frontal analysis of partially broken-down insulin (according to Sanger).

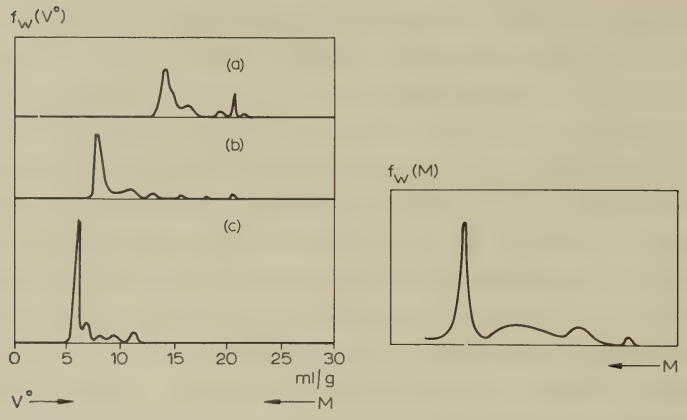


Fig. 17. Weight frequency curves of three different synthetic polymethylmetacrylates, obtained from frontal analysis curves (average molecular weight about 200,000). The curves give the frequency as a function of the adsorption (the retention volume). To the right is shown in comparison a curve for the preparation number 2, obtained from a number of ultracentrifugations on material which was fractionated by precipitation (Claesson).

for many proteins and similar substances. The adsorbent used in the column is a finely divided material which in water or weak salt solutions does not adsorb proteins or does so only very slightly. It has long been known that proteins can be « salted out » from solutions of, for example, ammonium sulphate in high concentrations. As this must depend on an increase of the thermodynamic activity of the protein molecules in the solution due to the salt, it must be expected that the adsorption will increase provided that the adsorbent is not influenced. In actual practice it is found that both with several proteins and a number of dye-stuffs and other salt-precipitable substances a strong adsorptive effect can be developed in this manner on various comparatively indifferent adsorbents, such as filter paper, starch, silica gel, etc., with salt concentrations essentially lower than is necessary for precipitation. The adsorption seems to be reversible and elution is accomplished by washing with water or with a weaker salt solution. Also by this means, the column is restored to its original condition and may be used again. An example of a separation by means of salting out adsorption on filter paper is shown in Fig. 18.

Salting out adsorption is shown by several proteins and even by some of their larger breakdown products. Also some viruses seem to show a pro-

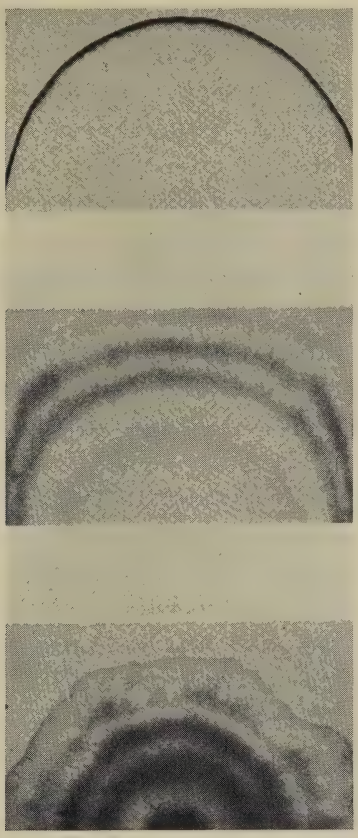


Fig. 18. The separation of dyes by salting out adsorption on filter paper. (*Top*): A mixture of naphthol green and fuchsin in water gives no separation. (*Middle and bottom pictures*): The same experiment in 1 molar and 2 molar ammonium sulphate solution gives a marked separation of the fuchsin (which is shown to contain three components) and naphthol green.

nounced effect. Up to the present, only intestinal virus from mice and from the foot-and-mouth disease have been tested, both with promising results.

It is perhaps too early yet to predict what practical value this method may attain – in conjunction with the recently described methods of observation. It is certain, however, that the need for specific and gentle methods of separation in the high molecular field is great, especially with respect to the enormous multitude of substances occurring in nature which are similar chemically but dissimilar with respect to function and activity.

Biography

Arne Wilhelm Kaurin Tiselius was born August 10, 1902, in Stockholm. After the early loss of his father, the family moved to Gothenburg where he went to school, and after graduation at the local «Realgymnasium» in 1921, he studied at the University of Uppsala, specializing in chemistry. He became research assistant in The Svedberg's laboratory in 1925 and obtained his doctor's degree in 1930 on a thesis «The moving-boundary method of studying the electrophoresis of proteins» (published in *Nova Acta Regiae Societatis Scientiarum Upsaliensis*, Ser. IV, Vol. 7, No. 4) and was appointed Docent (Assistant Professor) in Chemistry from 1930 on. During the years 1931–1935 Tiselius published a number of papers on diffusion and adsorption phenomena in naturally occurring base-exchanging zeolites, and these studies were continued during a year's visit to H. S. Taylor's laboratory in Princeton with support of a Rockefeller Foundation Fellowship. Stimulated by many contacts with American biochemists and physical chemists during this visit, Tiselius on his return to Uppsala resumed his interest in proteins, and the application of physical methods to biochemical problems in general. This lead among other things to a much improved method of electrophoretic analysis, published in the *Transactions of the Faraday Society*, 33 (1937) 524. This method as applied to the study of serum proteins and to a number of other biochemical problems kept Tiselius and an increasing number of collaborators occupied for the following years. In 1938 a special research professorship was established for Tiselius through a donation to the University of Uppsala by Major Herbert Jacobsson and his wife. Some space was put at the disposal of the new professor in the Institute of Physical Chemistry (Prof. The Svedberg). In 1946 biochemistry was established as an independent department and in 1950–1952 obtained a new building, the present Institute of Biochemistry.

Under the leadership of Tiselius this institute has contributed to the development and improvement of a number of useful methods in biochemistry, such as electrophoresis, chromatography, phase partition, gel filtration, etc. These methods and others have been applied to studies of large molecular

weight substances, chiefly proteins and enzymes, but also polysaccharides (dextran) and nucleic acids. There has always been a close contact between the methodological work and the research into special problems where the methods find their application. Tiselius took an active part in the reorganization of scientific research in Sweden in the years following World War II. Thus he was Chairman of the Swedish Natural Science Research Council 1946–1950, and Chairman of the Research Committee of the Swedish Cancer Society 1951–1955. He was President of the International Union of Pure and Applied Chemistry 1951–1955, became Vice President of the Nobel Foundation in 1947 and President since 1960. He has also served as member of the Nobel Committee for Chemistry since 1946.

He was married (1930) with Ingrid Margareta (Greta) Dalén, daughter of city judge Per Dalén of Gothenburg. They have two children: Eva (b. 1932), married to Dr. Torgny Bohlin, Lund; and Per (b. 1934), physician at the Academic Hospital, Uppsala.

Chemistry 1949

WILLIAM FRANCIS GIAUQUE

«for his contributions in the field of chemical thermodynamics, particularly concerning the behaviour of substances at extremely low temperatures»

Chemistry 1949

Presentation Speech by Professor A. Tiselius, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Royal Highnesses, Ladies and Gentlemen.

Chemistry has its origins far back in the cultural development of mankind. The search for the laws governing the structure of and changes in the material universe has followed many, now and then rather curious, paths. The alchemist's dream of making gold or of finding the philosopher's stone led to experiments which would be today regarded as unmethodical, but which nevertheless led gradually to a pretty thorough knowledge of the diverse properties of matter. The driving force behind this research was not only personal gain or a desire to please the aristocratic patrons who often had to pay for the experiments, but a genuine thirst for knowledge.

Chemistry became a science only when experiments became systematic and an attempt was made to discover general laws for the changes in matter. A scientific law should not only render possible a coherent account of all known natural phenomena, but should preferably enable us to predict new ones. When chemists today grope their way forwards in unknown fields, they must always, like the alchemists, try by means of experiments to determine what can and what cannot be done. But they are enormously helped by certain exact general laws which make it possible to predict the result of a chemical reaction under varying external conditions, for example different pressures and temperatures. To be able in this way to foresee the result of a chemical reaction is naturally of immense practical value. It is thus often possible nowadays to calculate in advance whether a given chemical process is possible and what conditions favour it. But such laws are also, of course, the basis of any comprehensive picture of the chemical processes in our world, so that research into them has occupied a central position ever since chemistry became a science.

The laws which I want particularly to touch on here are those dealing with the kinship between substances or, as chemists say, their affinity to one another, how this affinity shall be defined, how best to measure it and to what extent it can be calculated. When a lump of coal burns in air, heat is generated. The chemical reaction which takes place is that the coal combines

with the oxygen in the air to form carbon dioxide. It was natural that the quantity of heat thus generated was first of all thought to be a measure of the affinity – one would of course expect that the greater the tendency for the reaction to take place, the greater would be the heat. This is often but not always the case. Chemical reactions are known which cause a reduction in temperature. The heat generated during a reaction is not, therefore, suitable as an exact measure of the affinity. It has been discovered that we should measure instead the so-called free energy of the chemical reaction, i.e. the part of the total energy liberated during the reaction that can be used directly or indirectly for the production of mechanical or electrical energy. This free energy can be measured exactly by mechanical, electric or spectroscopic methods, but only under conditions which are normally not very easy to establish – it is necessary, for example, for the reaction to be studied in equilibrium. It is usually far simpler to measure the heat generated during a chemical reaction, the heat of reaction, and it is therefore natural that much research in this field has been devoted in the last fifty years to the problem of finding some way in which the free energy may be calculated from the heat, i.e. to enable us to predict the result of a chemical reaction from purely thermodynamic determinations.

One of the most brilliant scientists engaged in this research, and perhaps the foremost pioneer in chemical thermodynamics, was an American, Willard Gibbs, who worked at the end of the last century. He formulated clearly the equation which tells us that what we need to know, in order to calculate the free energy from the heat of reaction, is the change in *entropy* during the reaction. This change in entropy, multiplied by the absolute temperature, must be added to the heat of reaction in order to give the free energy.

This year's Nobel Prize winner, William Francis Giauque, has added considerably to our knowledge of the entropy of chemical substances, particularly at very low temperatures. I shall try to give you an idea of what entropy is, but first I must explain that one cannot observe it as one can, for example, temperature and pressure, and that even students of chemistry fight shy of this concept to begin with. If a substance is given a certain quantity of heat, then the entropy increases by an amount equal to the amount of heat divided by the absolute temperature. We can thus measure the change in entropy, and from these measurements obtain the data necessary for us to calculate the free energy and heat of reaction of a chemical reaction at a given temperature if we know them at another temperature. Entropy is an

extraordinarily interesting property of a substance, particularly in the light of the molecular and atomic theories. We thus know that what we call heat is the result of the motion of the molecules. A higher temperature corresponds to a more lively molecular motion. Entropy, again, is a measure of the state of molecular disorder. If we melt an ice crystal by heating it, the entropy increases by a quantity equal to the melting heat divided by the absolute temperature, and the almost perfect order of the water molecules in the ice crystals changes into the disorder prevailing in the water formed by the melted ice.

There is a general law that all spontaneous chemical and physical processes are associated with an increase in entropy. We must conclude, therefore, that the world is becoming more and more disordered – a conclusion which is naturally only proved for molecular processes.

There is another very important law stating that the entropy is zero for a crystallized substance at a temperature equal to the absolute zero, $-273.16°$.

This law – the third law of thermodynamics – first formulated by the German Nobel Prize winner, Walther Nernst, has only now been fully proved as a result of Giauque's work. By means of this law we can now calculate not only differences in entropy but the entropy itself for a considerable number of elements and chemical compounds. Thus, if we want to calculate the free energy necessary for the formation of, for example, a certain organic substance made up of carbon, hydrogen, oxygen, and nitrogen, we look up the entropies of these elements in a table and determine in addition the heat of formation and the entropy of the compound. The problem of calculating the chemical affinity from the heat of formation or the heat of reaction is therefore solved in principle.

Behind the data used in such calculations and behind the final proof of the third law lie, however, extensive studies of the properties of substances at temperatures approaching absolute zero. It is here that this year's Nobel Prize winner has made his greatest contributions to chemistry. By supreme experimental skill he has overcome the many great difficulties which must obviously be inherent in investigations under such extreme conditions. He has also worked out new methods for experiment in this field, of which his magnetic cooling method deserves special mention. This method has made it possible to reach temperatures nearer to absolute zero than was possible by any earlier technique.

Giauque's method is based on the fact that the crystals of certain substances (e.g. gadolinium sulphate) when magnetized at low temperatures, can be

brought to a higher degree of molecular order. Heat is liberated and led off in a bath of liquid helium at a temperature of 1° above absolute zero. After the liquid helium has been pumped away, so that the crystals are heat-isolated, the magnetic field is removed and the temperature falls to only some thousandths of a degree above absolute zero. The method is already widely used in a number of low-temperature laboratories in various parts of the world, and important discoveries in superconductivity and the magnetic properties of substances have been made with it. Giauque first mentioned the idea at a Congress of the American Chemical Society on the 9th of April, 1926. But not until 1933 did he have the necessary experimental resources to be able to realize his project. Characteristically enough, Giauque states himself that the first successful attempt was carried out between 3 a.m. and 9 a.m. on the morning of the 19th of March, 1933.

It should be mentioned that Giauque has also worked out important methods for measuring temperatures accurately just above absolute zero. In order to appreciate fully the importance of the fact that this temperature range has been opened up for research, one must understand that the properties of substances change just as much between 1° absolute and 0.003° absolute as they do from room temperature down to 1° absolute. Moreover, many natural phenomena become simpler when the motion due to heat has almost ceased and the molecules have lain down to rest. Giauque's contribution to chemistry has, thus, opened up a fascinating field for research where he, with his co-workers, has achieved most important results. In this entropy measurements he has succeeded in reaching an accuracy ten times greater than that of the best earlier measurements, thus creating the pre-requisite conditions for the fundamental results already mentioned: the proof of the validity of the third law and the accurate calculation of chemical equilibria. A number of different substances were used in these studies. I shall mention here only the beautiful investigation of the equilibrium between nitrogen oxides and between different hydrates of nitric acid. The investigations on the entropies of the pure metals are also of great interest. Determining the difference in entropy between the glass form and the crystalline form of glycerine – a problem which Giauque dealt with as long ago as in his doctor's thesis in 1923 – is particularly important in testing the third law.

Giauque has achieved many interesting results by comparing the entropy values he has obtained by these methods (calorimetric entropy) with the values he could calculate from the band spectra (spectroscopic entropy). The latter method was introduced by the Nobel Prize winner James Franck and

by the American physicist Birge. But Giauque has worked out practical methods for calculating the thermodynamic constants from the spectra. Here, as so often elsewhere in research work, a comparison between accurate data obtained by fundamentally different methods has given rise to interesting new discoveries. Giauque was able to explain the difference between the entropies thus found for carbon monoxide. The difference between the two ends of a carbon monoxide molecule is so slight that it can lie orientated in two directions in the crystal. Giauque has demonstrated this interesting effect in a number of substances with a greater or lesser high degree of symmetry in a series of papers published at the beginning of the thirties. These results, like so many others of Giauque's, are clear examples of the relationship between entropy and the degree of molecular disorder, a fact which I have just tried to explain.

In connection with such spectroscopic experiments, Giauque and Johnston made in 1929 the remarkable discovery that the element oxygen does not only consist of atoms with atomic weight 16 but contains in addition small quantities of oxygen isotopes with atomic weights 17 and 18. Since the atomic weight of oxygen is the basis for the calculations of the atomic weights of all the other elements, this discovery is of fundamental interest and also gave rise to similar investigations with other elements.

Giauque's achievements in the field of chemical thermodynamics and especially his work on the behaviour of matter at low temperatures and his closely allied studies of entropy comprise one of the most significant contributions to modern physical chemistry. The Royal Swedish Academy of Sciences derives great satisfaction from being able to reward this work with the Nobel Prize for Chemistry.

Professor Giauque. In order to extend our knowledge of those laws in Nature which determine the properties of matter and its transformations, it has been necessary to penetrate into the field of the lowest temperatures ever reached by man. Your brilliant achievements have been of decisive importance in such an approach. You have created methods necessary for accurate measurements under these extreme conditions, and you have applied these methods to a precise study of previously unknown phenomena which are of the deepest significance for science. Your results have afforded the final proof of one of the most fundamental laws in Nature, a law which is also of immense practical importance. It is with great satisfaction that the Royal Swedish Academy of Sciences has decided to award to you this year's Nobel

Prize for Chemistry. On behalf of the Academy I wish to extend to you our heartiest congratulations.

William Francis Giauque: May I now ask you to receive from the hands of His Royal Highness the Crown Prince, the Nobel Prize for Chemistry for the year 1949.

William F. Giauque

Some consequences of low temperature research in chemical thermodynamics

Nobel Lecture, December 12, 1949

The basic purpose which underlies most of the work in the Low Temperature Laboratory, of the University of California, is the study of entropy.

It is easy for a chemist to write an equation for a desired reaction, but this does not mean that the reaction will actually take place. If one knows the heats of formation and entropies of the substances concerned in the equation, the so-called free energy or thermodynamic potential of the reaction may be calculated. A knowledge of the free energy change permits chemists to determine all reactions which are thermodynamically possible and the extent to which they are possible. When it has been found that a certain reaction should go, but experiment fails, then a catalyst can be sought or the conditions can be altered so as to secure a practicable rate of reaction. If the free energy shows that the reaction is thermodynamically impossible, a search for a catalyst is futile.

The third law of thermodynamics, which developed from the Nernst Heat Theorem, states that all perfect crystalline substances approach zero entropy as the absolute zero of temperature is approached. According to this statement a knowledge of the heat capacity, to sufficiently low temperatures, will permit the calculation of the absolute entropy of a substance. It was this possibility which interested me in low temperature research.

Fig. 1 shows a few typical low-temperature heat-capacity curves for condensed gases. They have been measured by my students, Drs. R. Wiebe, H. L. Johnston, J. O. Clayton and R. W. Blue, and myself. The curve on the right of each graph represents the heat capacity of the liquid. The other curves represent the heat capacities of the one or more crystalline forms of the several substances.

Fig. 2 is a plot of the heat capacity of solid carbon dioxide against the logarithm of the temperature. According to the third law of thermodynamics the area under the curve, multiplied by 2.3026 to convert the ordinary to natural logarithms, is the absolute entropy of solid carbon dioxide.

Fig. 3 shows a drawing of the first low-temperature heat-capacity apparatus used in our work. It consisted of a calorimeter, C, surrounded by a protective hollow block of copper, B, both suspended in a high vacuum in container A.

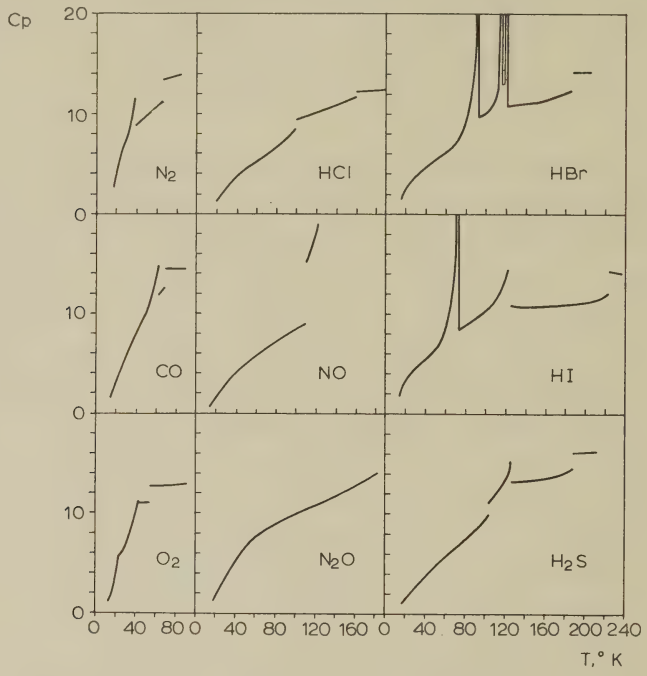


Fig. 1. Heat capacity in calories per degree per mole.

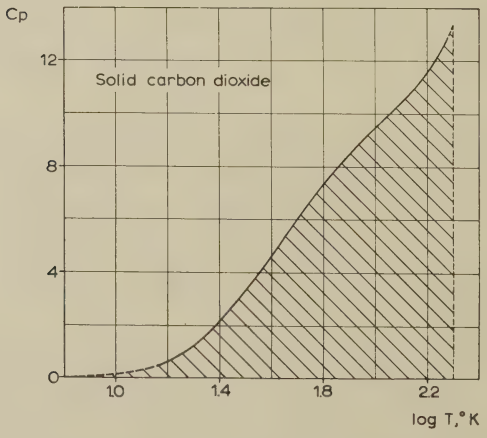


Fig. 2. Heat capacity in calories per degree per mole.

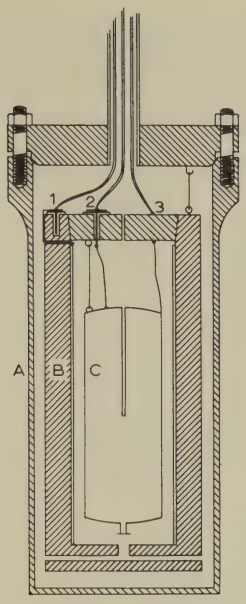


Fig. 3. Low temperature calorimeter.

The calorimeter was equipped with a resistance thermometer-heater, somewhat after the arrangement introduced by Professor A. Eucken. The block could be heated to, and held at, any obtainable temperature. In addition to resistance thermometers, copper-constantan thermocouples were attached to both calorimeter and block.

Fig. 4 shows a more recent calorimeter. It is of the type used for numerous low temperature investigations on condensed gases. The particular one shown was designed for the work with Dr. C. J. Egan on carbon dioxide which is illustrated in Fig. 2. The protective hollow block contains considerable lead in the upper portion to maintain an appreciable heat capacity at the lower temperatures. The apparatus can be cooled, first with liquid nitrogen, and then with liquid and solid hydrogen, until it reaches a temperature near 10° absolute. Helium gas is used in the space surrounding the calorimeter to provide heat conductivity during the cooling. When the cooling is completed the helium is removed by means of a high-vacuum pump and measurements are started. Some idea as to the sensitivity of the resistance thermometers used in this work may be obtained from the fact, that there are approximately 10,000 millimeter marks for each degree centigrade, on the scale which is read. If desired, this may be increased to the order of 30,000

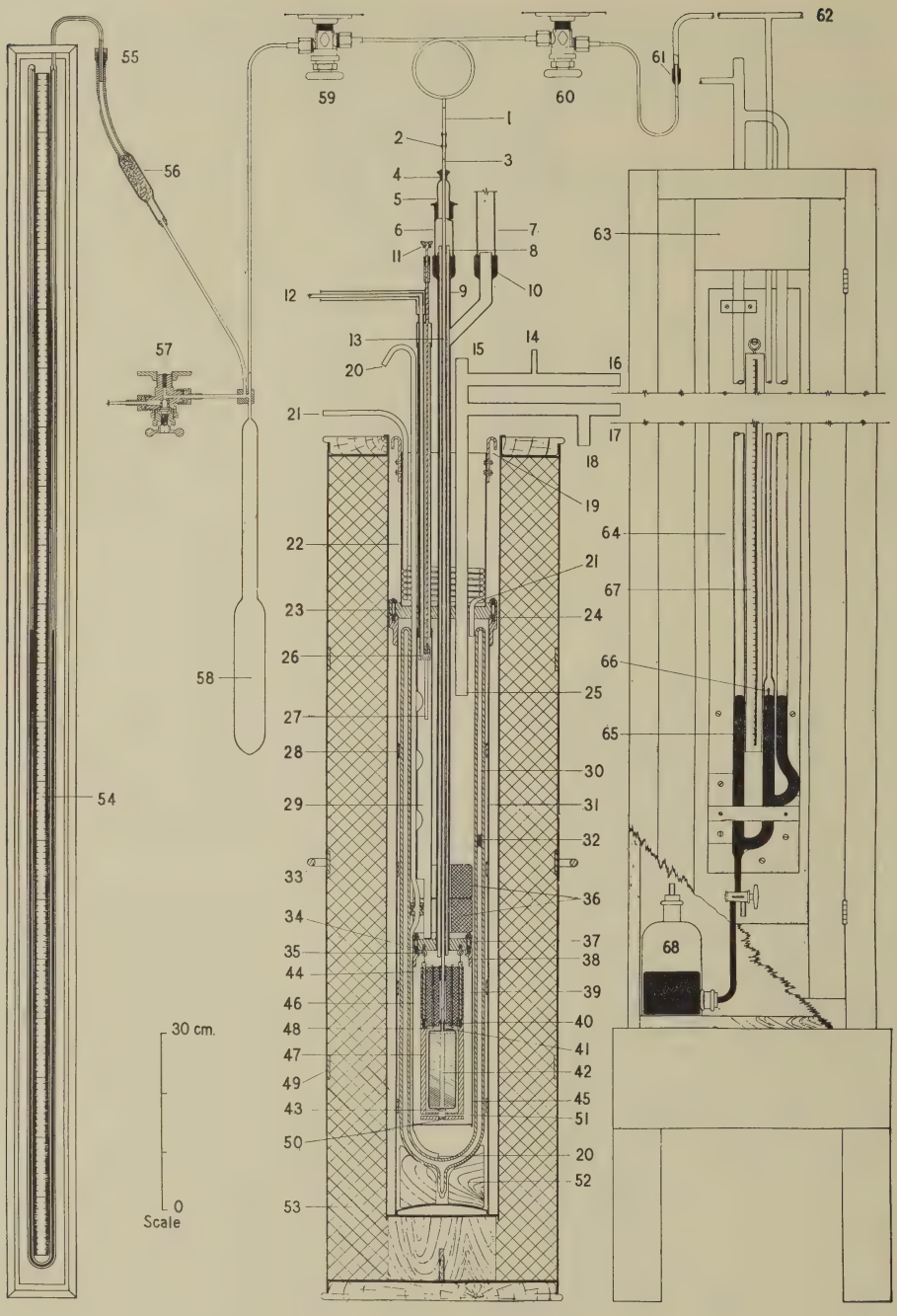


Fig. 4. Low temperature calorimeter for condensed gases.

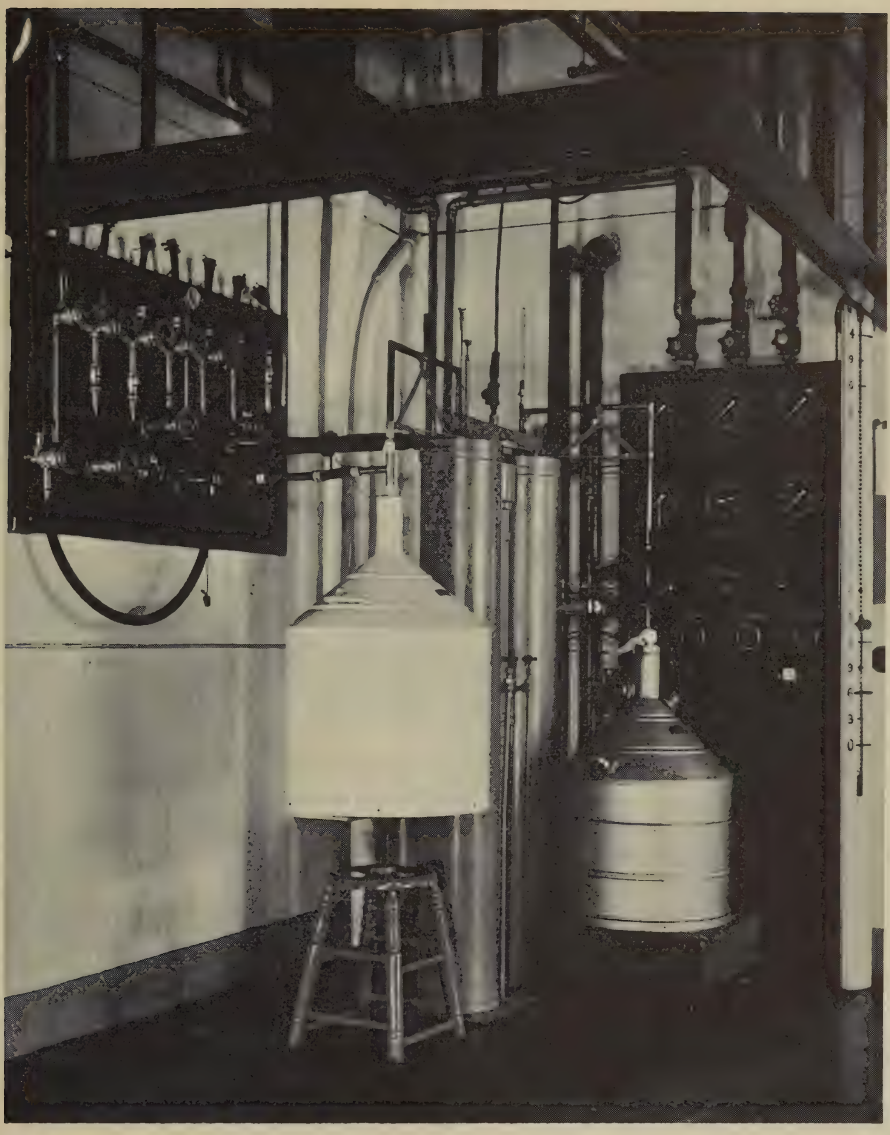


Fig. 5. Hydrogen liquefier.

millimeters per degree, for much of the range below 100° K. This high precision is desirable for observing heat leak and the attainment of thermal equilibrium within the calorimeter.

Figs. 5 and 6 show typical views in the low temperature laboratory. Fig. 5 is a photograph of the hydrogen liquefier located under a ventilating hood.

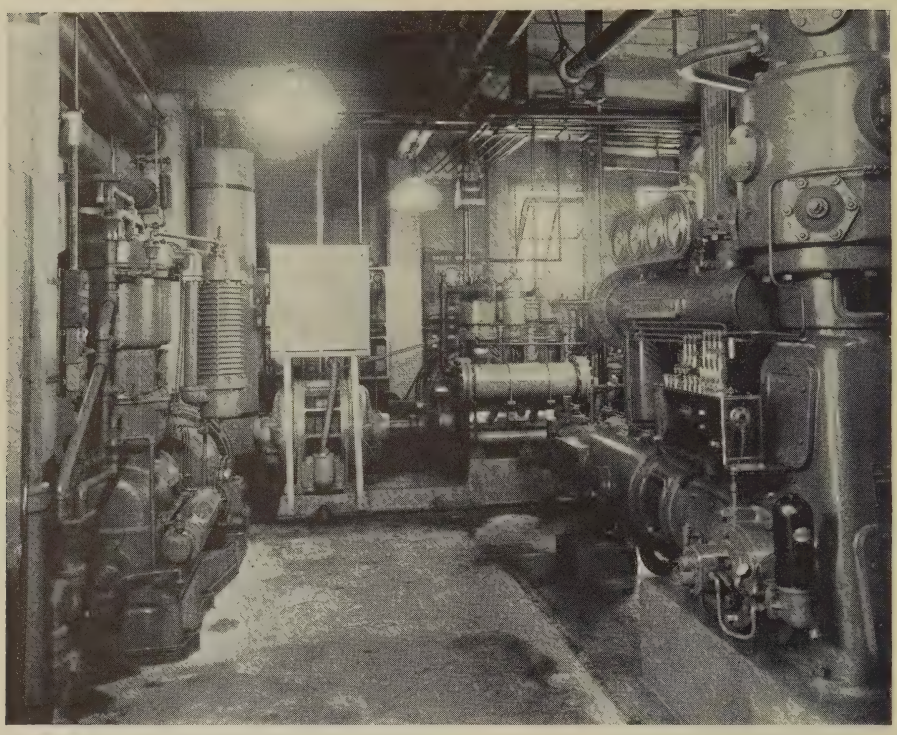


Fig. 6. View in machinery room. Ethane compressor (*left*), hydrogen compressor (*center*), air compressor (*right*).

A container of liquid nitrogen, for cooling the high-pressure hydrogen gas, stands at the right of the liquefier, and a similar 50 liter container is attached to a vacuum-jacketed transfer tube, to receive the liquid hydrogen.

Fig. 6 shows a portion of the machinery room with the compressors used in connection with the liquefaction of ethane, hydrogen, and air.

Practically all of the equipment in the laboratory has been specially designed for its purpose and, aside from compressors and standard instruments, it has been largely constructed in the laboratory shops. Fig. 7 is a photograph of a heat interchanger with a special type of construction that we have developed for this work. The one shown is used to liquefy air. Purified air at 270 atmospheres pressure is sent through the interchanger tubes, and after being pre-cooled with liquid ethane, some 30 percent of the air is obtained as liquid by means of simple Joule-Thompson expansion. A little over one liter of liquid is produced for each kilowatt hour of energy. The liquid can be withdrawn as liquid air, but is normally separated into pure nitrogen (0.03%

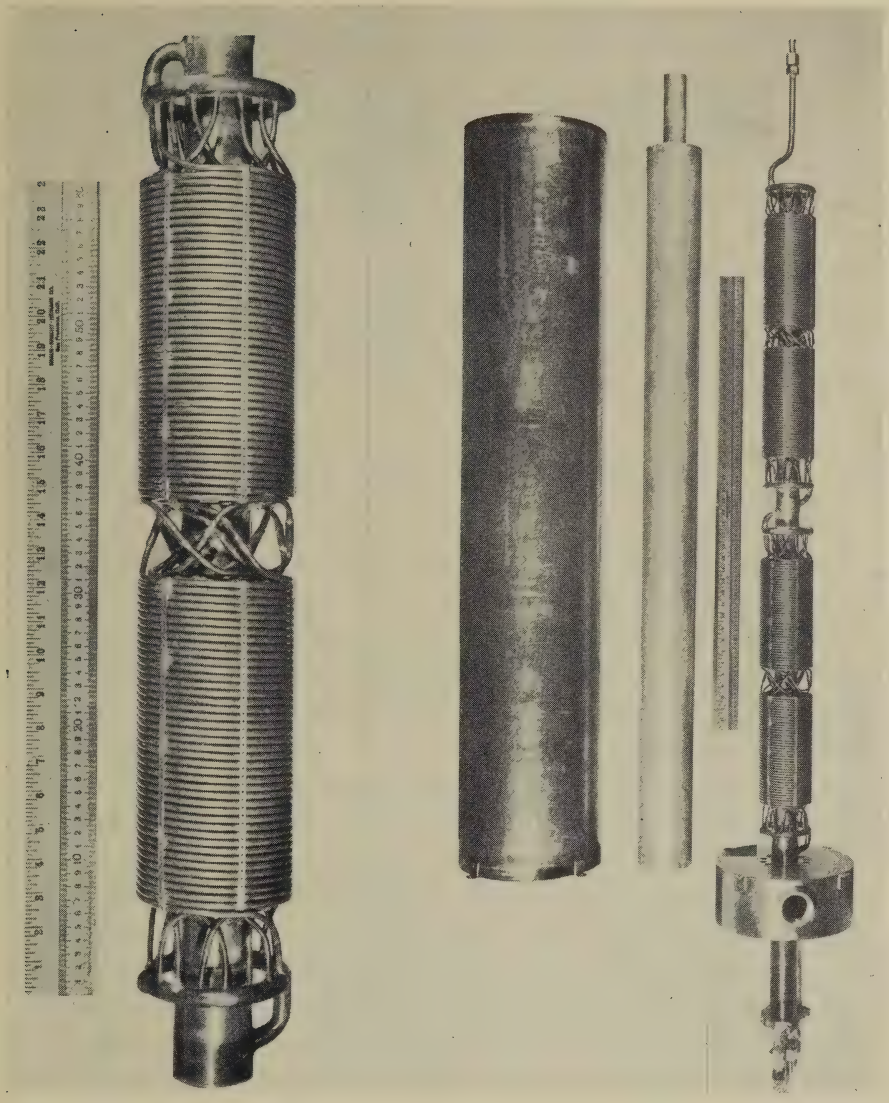


Fig. 7. High-pressure heat interchanger.

oxygen) and oxygen (99.8 to 99.5% depending on the amount produced) before their simultaneous withdrawal as liquids.

The interchanger shown in Fig. 7, was designed to produce 70 liters of liquid per hour. An enlarged view of a portion of it is shown at the left. The complete interchanger with its jacket and insulating case is shown at the right.

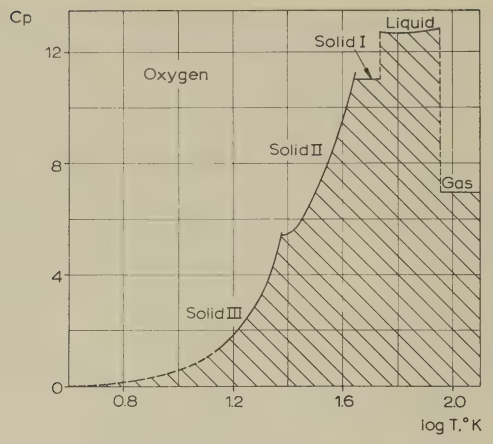


Fig. 8. Heat capacity in calories per degree per mole.

The equipment which has been described has been used to determine the entropies of many chemical substances.

The careful determination of entropy has often been accompanied by the discovery of unexpected physical properties. The discovery of the oxygen isotopes of atomic weights 17 and 18 was a case of this kind. The sequence of events was as follows:

An accurate measurement of the low-temperature heat capacity of oxygen was undertaken with Dr. H. L. Johnston. The data are represented in Fig. 8, where the heat capacity has been plotted against the logarithm of the absolute temperature. The area under the curves was used to calculate the entropy of oxygen gas. In this case, as had long been known, there are three crystalline forms and the liquid before the gas state is reached and thus it was also necessary to measure the increase in entropy during each change of state.

One of the principal lines of research which we have pursued in connection with the determination of entropy is the calculation of this, and other thermodynamic quantities, by means of quantum statistics and the energy levels of gas molecules. These energy levels can be obtained from band spectra.

Fig. 9 is a photograph of the band spectrum of oxygen taken by Mr. Harold D. Babcock of Mount Wilson Observatory. The strong doublets are due to ordinary oxygen (16–16) and their interpretation is due to Professor R. S. Mulliken of the University of Chicago.

This spectrum permits the determination of the energy levels of the oxygen molecule. When the entropy of oxygen gas was calculated from these strong

lines it agreed exactly with the value obtained from our low temperature measurements.

The spectrum also contains many weak lines, which were believed to be due to oxygen, but which were not understood. These weak lines were discovered by Babcock and most of them were measured and published by Dieke and Babcock. Later Babcock published some additional observations.

It is rather interesting that these weak lines are themselves an unexpected by-product of a solar investigation by Babcock. When sunlight passes through the molecules in the earth's atmosphere some of the light is absorbed selectively by them. This effect may be enhanced by photographing the sun when it is low on the horizon because the light then passes through a greater amount of air.

An entropy calculation based on band spectra is not considered to be satisfactory unless the spectrum is completely explained. One way to explain weak lines is to assume, that they are due to some higher energy state of the molecule, and are weak because not many molecules are in the higher energy state. Many of the weak lines in the oxygen spectrum are actually due to this effect but they could not all be explained in this way.

Months of thinking about this problem led to memorization of the essentials of the data and I literally awoke one morning with the realization that the lines must originate from an isotopic species. Detailed calculations by Dr. Johnston and myself confirmed this accurately and it was determined that isotopes of atomic weights 17 and 18 exist in the earth's atmosphere.

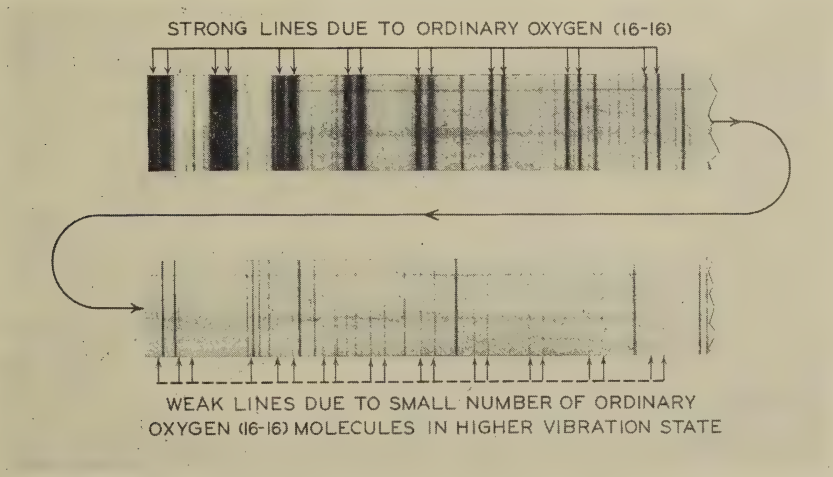

STRONG LINES DUE TO ORDINARY OXYGEN (16-16)
WEAK LINES DUE TO SMALL NUMBER OF ORDINARY
OXYGEN (16-16) MOLECULES IN HIGHER VIBRATION STATE

Fig. 9. Small section of band spectrum due to sunlight absorbed in Earth's atmosphere.

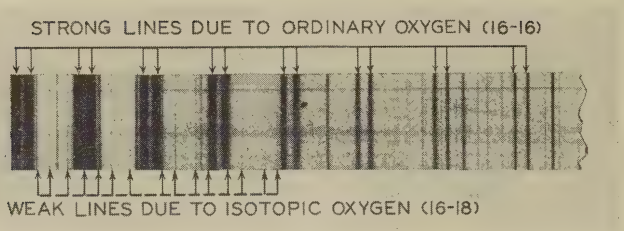


Fig. 10. Small section of atmospheric oxygen bands showing lines due to oxygen 16–18. (*Photograph by Harold D. Babcock, Mount Wilson Observatory.*)

In Fig. 10 the photograph of the oxygen band spectrum is shown with arrows pointing to the weak lines due to the 16–18 oxygen molecules. A similar set of very faint lines, which are not visible in Fig. 10 are due to molecules of oxygen 16–17.

When suitable isotopic masses are assumed, all of the weak lines due to isotopes can be calculated accurately from the positions of the strong lines.

The discovery that oxygen isotopes existed made it evident that physicists and chemists were unknowingly using different scales of atomic weights. Chemists take the atomic weight of the isotopic mixture as 16. Physicists use a mass spectrograph and take the predominant isotope as 16. This isotope is somewhat less than 16 on the chemists' scale.

Similarly, the adiabatic demagnetization method of producing low temperatures, was an unexpected by-product of our interest in the third law of thermodynamics.

As we have seen, the heat capacities of substances ordinarily become very small at temperatures below 10 or 15° absolute. Thus it had been considered that essentially all of the entropy had been removed from substances at these low temperatures and, aside from a minor extrapolated amount, it was customary to assume this in calculating entropy.

During a seminar in the fall of 1924 I presented calculations showing the way in which magnetic fields affect the thermodynamic properties of various substances. Some magnetic susceptibility measurements on gadolinium sulfate octahydrate, at the temperature of liquid helium, came to my attention. These measurements had been published by Professors Woltjer and Kamerlingh Onnes, from the University of Leiden.

The measurements of Woltjer and Kamerlingh Onnes are shown in Fig. 11. By means of appropriate thermodynamic equations it was possible to calculate the change of entropy when a magnetic field is applied. I was greatly

surprised to find, that the application of a magnetic field removes a large amount of entropy from this substance, at a temperature so low that it had been thought that there was practically no entropy left to remove.

The above conclusion was based only on the application of thermodynamics to the experimental data; however, further investigation led to a quantum statistical explanation of the magnetic data. The curve in Fig. 11 is not drawn through the points but is the result of a calculation entirely independent of the measurements.

Fig. 12 illustrates what happens when a magnetic field is applied to a paramagnetic substance like gadolinium sulfate. The arrows on the diagram correspond to atomic magnets. Their normal state is one of disorder which corresponds to the presence of entropy. When a sufficiently powerful magnetic field is applied the magnets line up and the entropy is removed. The removal of entropy is accompanied by the evolution of heat. Those familiar with thermodynamics will realize that in principle any process involving an entropy change may be used to produce either cooling or heating. Accordingly it occurred to me that adiabatic demagnetization could be made the basis of a method for producing temperatures lower than those obtainable with liquid helium. Professor P. Debye also arrived at similar conclusions.

In order to understand refrigeration by means of magnetic properties we will look at the analogous case of refrigeration by means of a gas expansion engine in an idealized form.

The first step is shown in Fig. 13. It illustrates the first part of the compression of a gas in a cylinder from which heat can escape. The entropy of the gas is decreased.

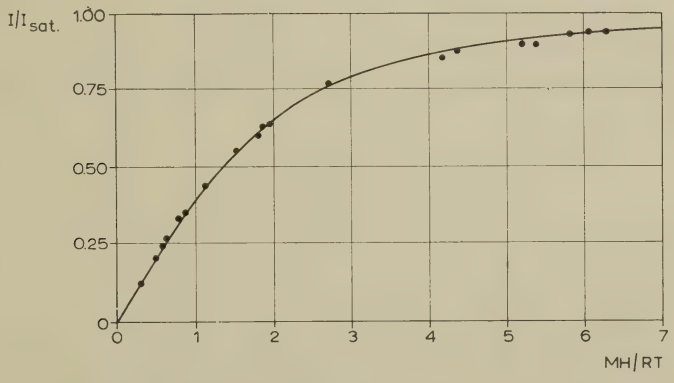


Fig. 11. Intensity of magnetization of $Gd_2(SO_4)_3 \cdot 8H_2O$. Data of Woltjer and Kamerlingh Onnes compared with the theoretical curve.

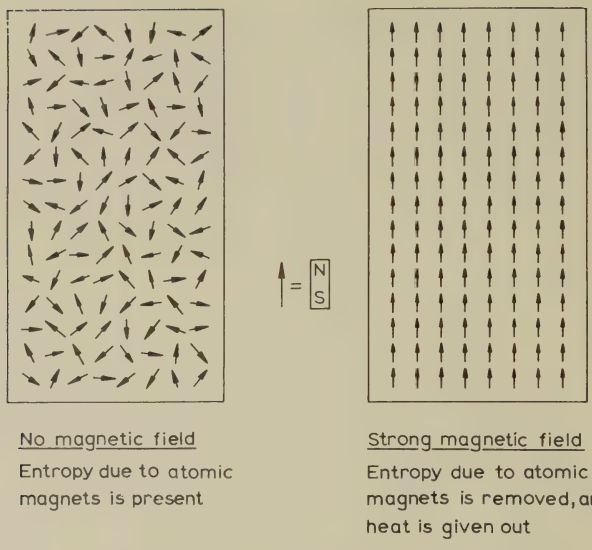

= N S
No magnetic field
Entropy due to atomic magnets is present
Strong magnetic field
Entropy due to atomic magnets is removed, and heat is given out

Fig. 12. Atomic magnets in crystal lattice.

The second step is shown in Fig. 14. The compression has been ended and heat has been given out so that the compressed gas has the same temperature as it had before compression. Some heat insulation is now placed symbolically so that no heat can pass between the gas and its surroundings.

The third and final step is shown in Fig. 15. The gas is permitted to expand and does work against the piston. The energy equivalent of this work comes from the thermal energy of the gas molecules and thus the gas cools. This process is called adiabatic expansion.

The steps leading to adiabatic demagnetization are very similar. Figure 16 is a schematic drawing showing a paramagnetic substance located within the coil of a solenoid magnet. The magnetic material is enclosed in a jacket, which is filled with helium gas to conduct heat. The apparatus inside the coil is immersed in liquid helium. When the current is started through the coils

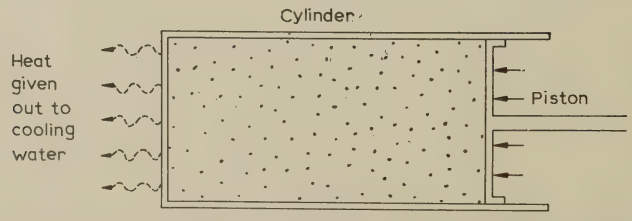


Fig. 13. Refrigeration by means of an expansion engine (idealized). *First step:* Gas is compressed. Heat is given out.

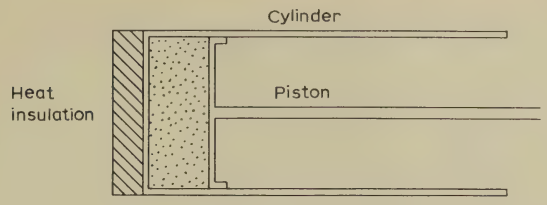


Fig. 14. Refrigeration by means of an expansion engine (idealized). *Second step:* Compression complete. Heat has been removed until final temperature equals initial temperature. Cylinder is then insulated against flow of heat.

of the magnet the atomic magnets begin to line up, heat is given out, and the entropy is decreased. The magnet coil in Fig. 16 appears to be in the liquid helium region; however, it is not placed there in actual experiments for practical reasons.

The second step is shown in Fig. 17, which illustrates the limiting case of complete magnetization. Heat has escaped until the final temperature is the same as the initial temperature. In actual experiments the magnetization is often very far from complete due to the limitations of equipment.

The substance is now insulated against heat flow by evacuating the helium gas from the insulating jacket.

The third, and final, step is shown in Fig. 18. As the magnetic field is decreased the magnetic material does work by contributing to the current in the surrounding circuits. This work is analogous to that done on the piston of the expansion engine but in this case it is done through the agency of electromagnetic fields acting through space. The work is done at the expense of the thermal energy of the substance which is thus cooled to a very low temperature.

The fact that energy can be removed from a paramagnetic substance, through a highly evacuated space, by means of electromagnetic work, is of

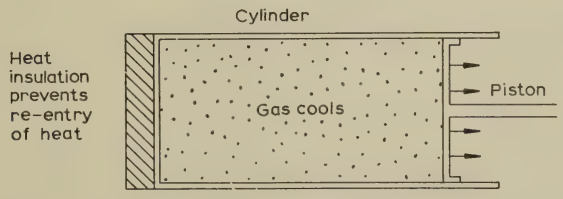


Fig. 15. Refrigeration by means of an expansion engine (idealized). *Third step:* Gas expands. No heat can enter. Mechanical work is done on the piston at the expense of the molecular energy. Loss of energy cools gas to lower temperature. This is adiabatic expansion.

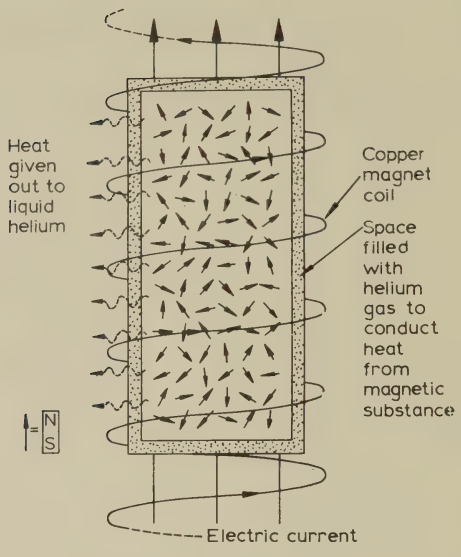

Heat given out to liquid helium
Copper magnet coil
Space filled with helium gas to conduct heat from magnetic substance
N
S
Electric current

Fig. 16. Refrigeration by means of adiabatic demagnetization. *First step:* Electric current is started through magnet. Heat is given out as paramagnetic substance is magnetized.

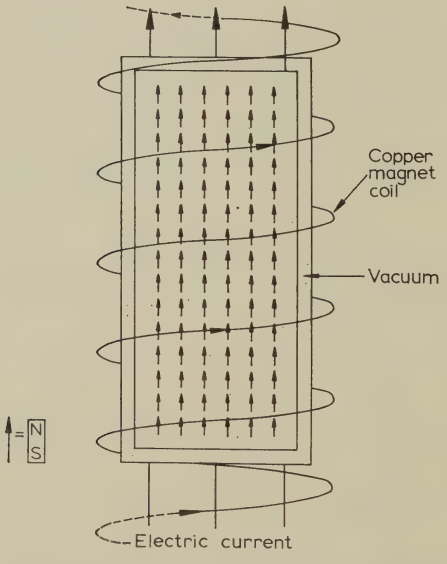


Fig. 17. Refrigeration by means of adiabatic demagnetization. *Second step:* Full electric current through magnet. Magnetization is complete. The substance is now insulated against flow of heat by pumping a vacuum in the jacket.

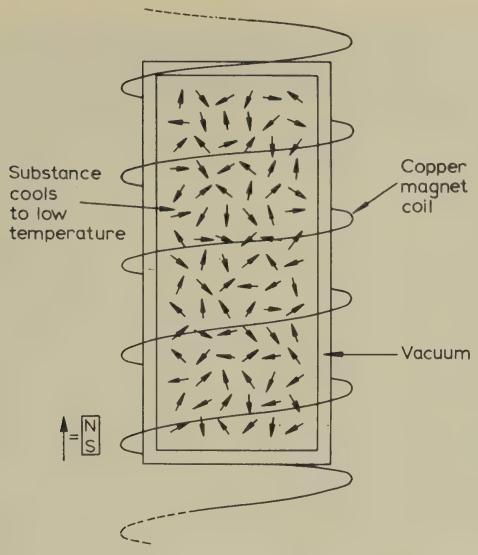


Fig. 18. Refrigeration by means of adiabatic demagnetization. *Third step:* Electric current turned off. Substance is demagnetized. No heat can enter. Substance does magnetic work through space by inducing an electric current in copper coils of magnet. The work is done at the expense of the molecular energy. Loss of energy cools substance. This is adiabatic demagnetization.

great practical importance to the method. It is this fact that makes possible the almost complete thermal isolation of the working substance while the low temperature is being produced.

Fig. 19 shows a drawing of an early apparatus. The central tube is filled with a paramagnetic substance. When the magnetic field is applied, the heat evolved boils away some of the liquid helium. The jacket, which can be evacuated after the heat is removed, surrounds the magnetic material. The low temperature is produced as soon as the magnet current can be turned off. A coil of tubing is shown around the outside of the Dewar vessel. Liquid nitrogen is passed through this coil to protect the liquid helium from radiation heat leak.

Fig. 20 shows a drawing of the solenoid magnet which has been used in this work. Sections have been shown as though they were cut away to disclose the conductors and the interior. The adiabatic demagnetization apparatus is mounted in the center. Cooling oil is pumped rapidly over the bare copper conductors to remove heat. Efficient heat transfer is the principal problem in the design of such magnets.

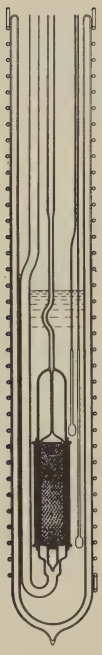


Fig. 19. Paramagnetic substance in vacuum jacket immersed in Dewar vessel of liquid helium.

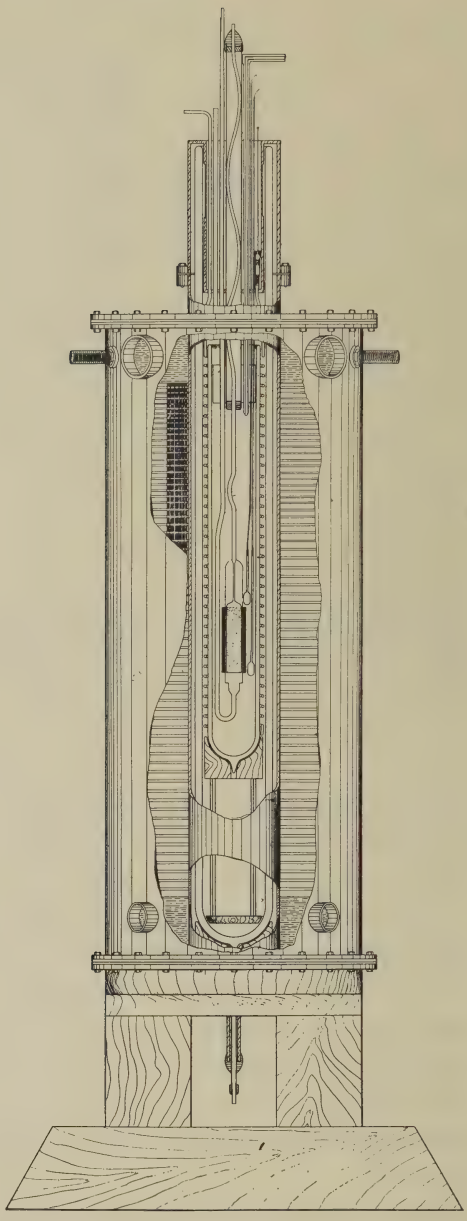


Fig. 20. Solenoid magnet with adiabatic demagnetization apparatus.

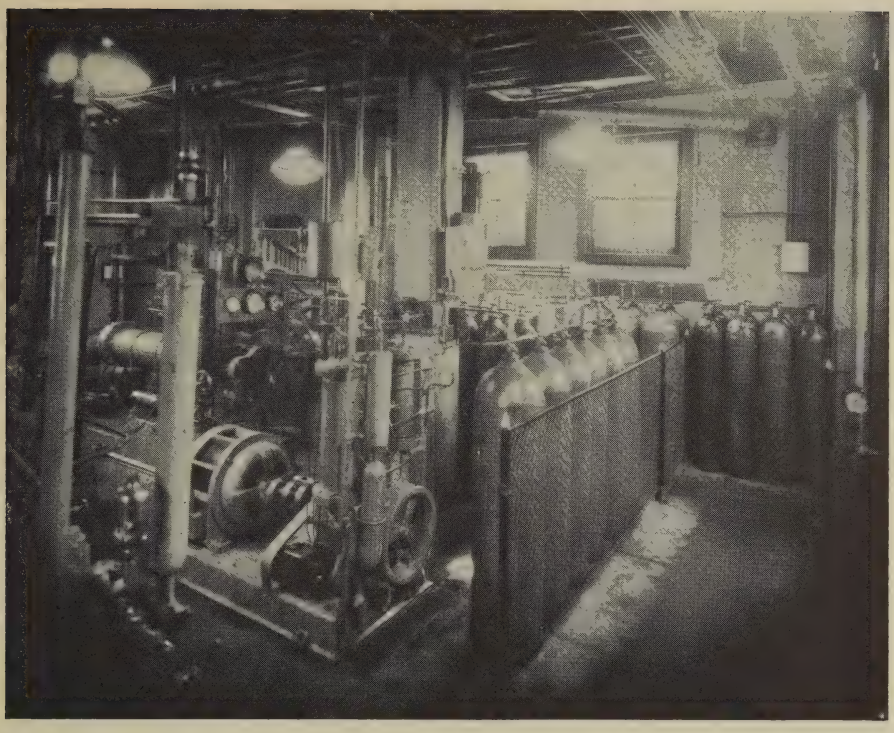


Fig. 21. View in machinery room. Helium compressor and gas storage cylinders in foreground.

Some additional photographs of the low temperature laboratory are shown as Figs. 21, 22 and 23. Figure 21 shows the helium compressor and some of the gas storage cylinders. Figure 22 shows the helium liquefier to the right of the solenoid magnet. Liquid helium is transferred through a vacuum jacketed transfer tube into the Dewar vessel in the interior of the magnet. In our present arrangement the liquid helium can be made about one meter deep. Additional liquid is added only once a day when experiments of long duration are in progress.

Fig. 23 is a photograph of the apparatus about as it was assembled and used by Dr. D. P. MacDougall and myself in the first adiabatic demagnetization experiment in 1933. The inductance bridge used to measure the magnetic susceptibility of the gadolinium sulfate octahydrate used is shown on the table in the foreground.

MacDougall sat at the table to make the first observation, I pulled the

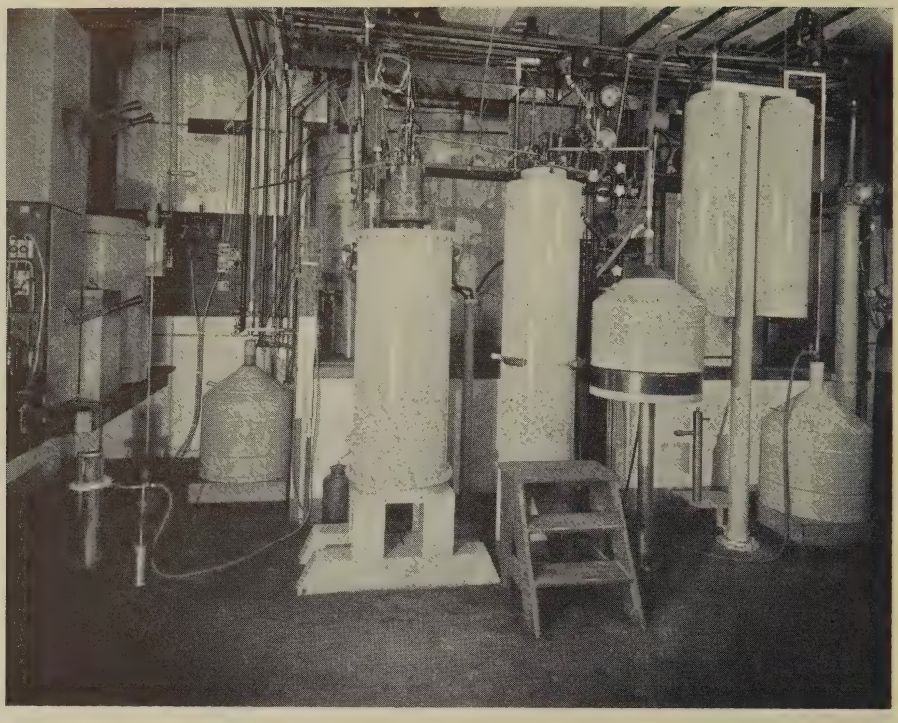


Fig. 22. Helium liquefier (*behind steps*), helium purifier (*at right*), solenoid magnet (*at left*).

switches in the left background and watched the expression on MacDougall's face. In a short time he announced that the cooling had occurred.

The commonest question asked in the early days of this work was: « How do you know it gets cold? » This was a fair question. Obviously no one had ever made thermometers which were calibrated at temperatures that had never been produced. Since even helium gas has negligible pressure, at the low temperatures obtained, a gas thermometer is useless.

Temperature can only be measured by some property of a substance which varies with temperature. In this case the magnetic susceptibility increases as temperature decreases.

Fig. 24 illustrates the measuring coil system of an early apparatus. The coil is in several sections, two of which are around the equatorial region of the simple cylindrical sample to minimize correction for end effects. The coil system is outside the vacuum jacket and is immersed in liquid helium during use. The coils contained many thousands of turns of fine copper wire. When

an alternating electric current is passed through a measuring coil it becomes increasingly difficult for the current to pass as the magnetic susceptibility of the substance increases. This effect permits the quantitative determination of magnetic susceptibility.

Fig. 25 shows how the magnetic susceptibility varies with temperature according to Curie's law. However, one of the consequences of the third law of thermodynamics is that Curie's law must fail as the absolute zero is approached. Curie's law has proved very useful but temperatures obtained in this way are at best approximate.

Lord Kelvin defined thermodynamic temperature in such a way that any method utilizing an entropy change to attain a lower temperature, contains within itself, a method of determining that temperature. One must, of course, be able to make the necessary measurements in a thermodynamically reversible manner. Fortunately this is a straight forward procedure in the case of many paramagnetic substances although it requires a large number of correlated experimental measurements.

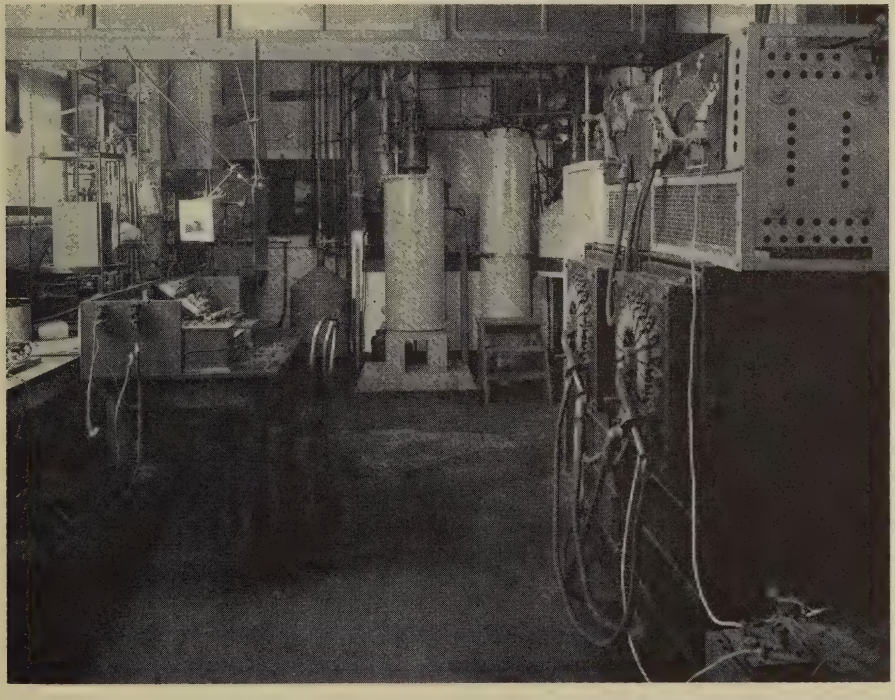


Fig. 23. Inductance bridge (*on table*). Solenoid magnet and helium liquefier (*centre rear*).

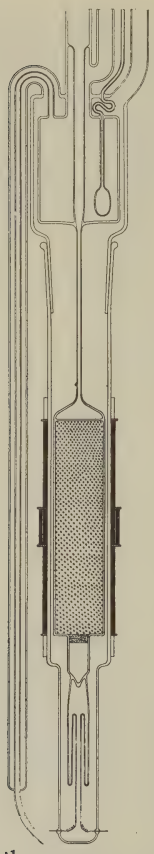


Fig. 24. Measuring coil system around paramagnetic sample.

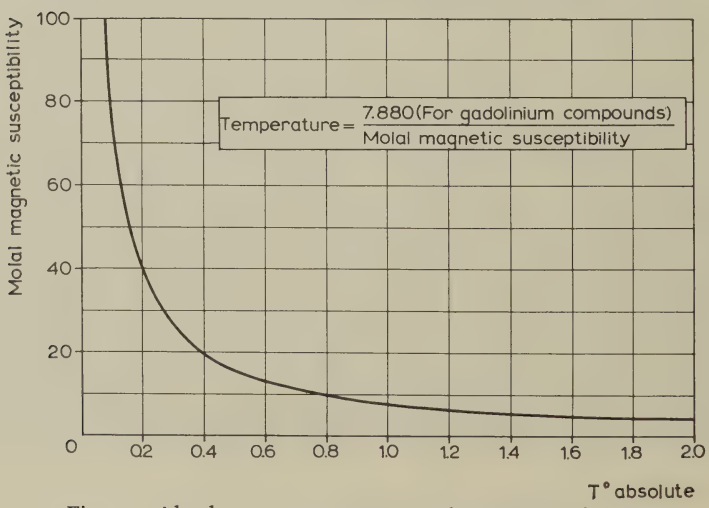


Fig. 25. Absolute temperature according to Curie's law.

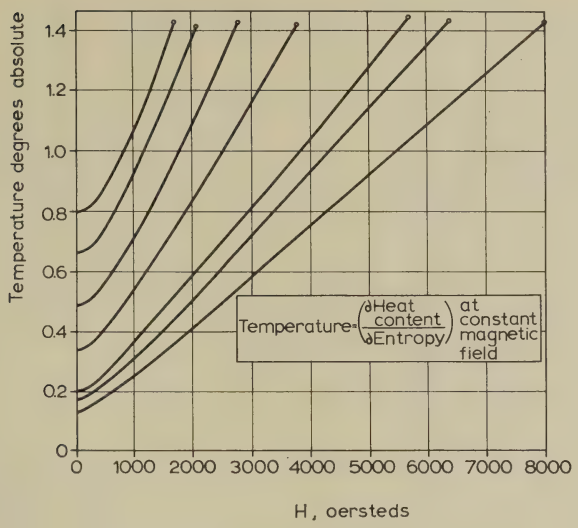


Fig. 26. Change of thermodynamic temperature with magnetic field at constant entropy for gadolinium phosphomolybdate tridecahydrate.

Fig. 26 shows the way in which the true absolute temperature changes during the course of adiabatic demagnetization of the chemical compound, gadolinium phosphomolybdate tridecahydrate. These temperatures were calculated from the thermodynamic relationship that the absolute temperature is equal to the rate of change of heat content with entropy at constant magnetic field.

It may be seen that there is a definite lower limit of temperature which is extended downward by increasing the initial magnetic field. The field available in our present magnet is only 8,000 oersteds, however it is expected that much more powerful magnets will be available in the not too distant future.

As is well known a temperature of 0.004° K has been produced in this way with a field of 24,000 oersteds by Professors de Haas and Wiersma, at the University of Leiden.

Fig. 27 shows the characteristics of the most sensitive type of thermometer we have devised for use in the region below 1° absolute. It was made of lampblack, supported on optical polishing paper, cemented together and held on to glass, by means of a very thin film of collodion. The data shown were obtained during work with Drs. J. W. Stout and C. W. Clark.

Resistance thermometers of this type are also useful as electric heaters for introducing measured quantities of heat under some circumstances. Long

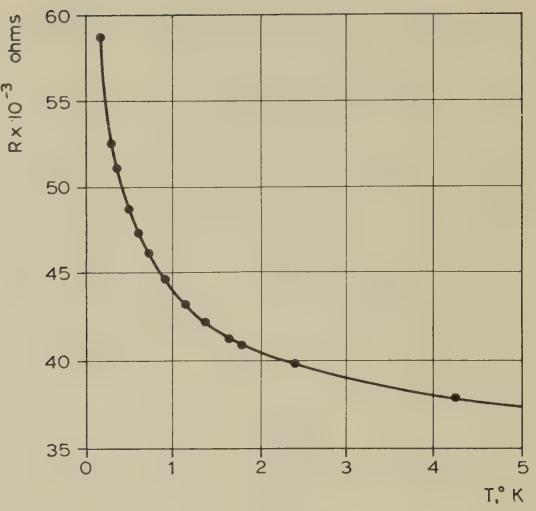


Fig. 27. The resistance of a carbon thermometer as a function of temperature.

glass leads with narrow platinized strips to carry the current are shown near the bottom of Fig. 24.

An example of the measurement of heat capacity in this way, to temperatures below 1° K, is shown in Fig. 28, for cobalt sulfate heptahydrate. The apparatus utilized by Dr. J. J. Fritz to measure the heat capacity and magnetic properties of $CoSO_4 \cdot 7H_2O$ is shown in Fig. 29. The ellipsoidal

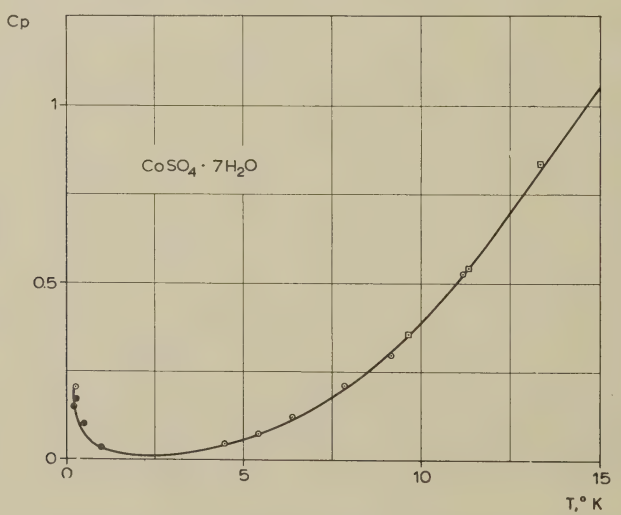


Fig. 28. Heat capacity in calories per degree per mole.

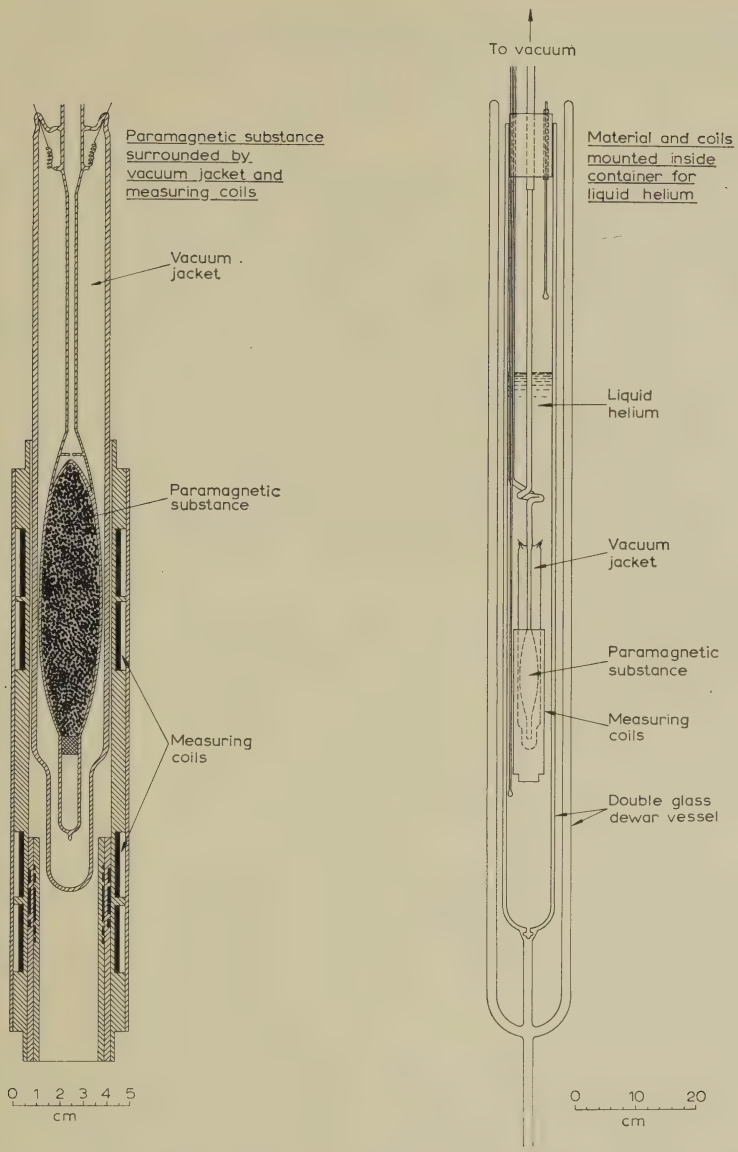


Fig. 29. Adiabatic demagnetization apparatus.

shape simplifies magnetic behavior and makes accurate calculation of end effects possible. Magnetic hysteresis can be investigated with any of the calorimeters which have been shown, by means of the heating effect in an alternating field. With 60 cycle current the hysteresis in gadolinium compounds is undetectable, except at the very low temperatures, and even there

it is so small, that the processes of magnetization and demagnetization rank with the most perfectly reversible of known processes. However, this is not true in all substances and all sorts of effects ranging to remanent magnetism exist.

If a substance becomes a permanent magnet at very low temperatures, it is easy to measure the strength of the magnet. This may be done by slowly warming the magnetic material. The apparatus shown then acts as an electric generator with no moving parts except the atoms. As the paramagnetic substance is warmed, any change in the magnetic moment will generate a potential in the coil, which can be quantitatively measured by a sensitive galvanometer and used to determine the total magnetic induction.

The resistance of the wire in measuring coils drops to such low values at the temperatures of liquid helium that very large numbers of turns can be used. The sensitivity which is obtained with coils at these low temperatures is such that it is necessary to compensate for the small fluctuations in the Earth's magnetic field.

These are some examples of the type of things that are to be found by those who inquire into the subject of entropy. We consider it a rich field for further investigation.

Biography

William Francis Giauque was born in Niagara Falls, Canada, of U.S. parentage, on May 12, 1895, the first of three children of William Tecumseh Sherman Giauque and Isabella Jane Duncan.

He attended public grammar schools principally in Michigan. Following the death of his father, in 1908, the family returned to Niagara Falls, Canada, where he received his secondary school education in the Niagara Falls Collegiate Institute. After graduation he sought employment in various power plants at Niagara Falls for financial reasons and because he had planned for many years to become an electrical engineer and wanted preliminary experience. He was unable to obtain this type of work. At this point chance entered decisively in the form of a newspaper advertisement of the Hooker Electro-Chemical Company in Niagara Falls, New York, which led him to accept employment in their laboratory. The well-organized operations in this chemical plant, together with problems which he saw in course of solution, captured his interest and caused him to decide to become a chemical engineer.

After two years employment he entered the College of Chemistry of the University of California, where he received the B. S. degree with highest honors in 1920, was a University Fellow for the year 1920–1921 and James M. Goewey Fellow 1921–1922. He received the Ph. D. degree in chemistry with a minor in physics in 1922.

Although his undergraduate work at the university was selected with the idea of an engineering career, he soon acquired a liking for fundamental research. The emphasis on scientific investigation by the group of faculty and students associated with Professor Gilbert N. Lewis was the major influence.

He was appointed Instructor of Chemistry in 1922 and after passing through the intermediate grades of professorship he became Professor of Chemistry in 1934.

His interest in the third law of thermodynamics as a field of research was aroused by the experimental work for his Ph. D. research under Professor

G. E. Gibson. This work, which was concerned with the relative entropies of glycerine crystals and glass, had its origin in discussions of Professors Lewis and Gibson.

The principal objective of his researches has been to demonstrate through a considerable number and variety of accurate tests that the third law of thermodynamics is a basic natural law.

The researches of his students and himself have included a large number of accurate entropy determinations from low temperature measurements, particularly on condensed gases. The entropies and other thermodynamic properties of many gases have also been determined from quantum statistics and molecular energy levels available from band spectra and other sources.

Correlated investigations of the entropy of oxygen from its incompletely interpreted band spectrum and from low-temperature heat capacity measurements with Dr. H. L. Johnston, led to the discovery of oxygen isotopes 17 and 18 in the Earth's atmosphere and the fact that chemists and physicists were unknowingly using different atomic weight scales.

Investigation of the effect of magnetic fields on the entropies of paramagnetic substances led to the invention of the adiabatic demagnetization method of producing temperatures considerably below 1° absolute. This method was first demonstrated in collaboration with Dr. D. P. MacDougall.

Professor Giauque's scientific work has been described in some 75 papers.

He has received the Chandler Medal and the honorary degree of Sc. D. from Columbia University, an honorary LL.D. from the University of California, and the Elliott Cresson Medal from the Franklin Institute. In 1951 he received the Willard Gibbs Medal and in 1956 the Gilbert Newton Lewis Medal. He was Faculty Research Lecturer of the University of California in 1948. He is a member of the National Academy of Sciences, the American Philosophical Society, the American Chemical Society, Institut International du Froid, and is Fellow of the American Physical Society and of the American Academy of Arts and Sciences.

In 1932 he married Dr. Muriel Frances Ashley. They have two sons, William Francis Ashley G. and Robert David Ashley G.

Chemistry 1950

OTTO PAUL HERMANN DIELS

KURT ALDER

«for their discovery and development of the diene synthesis»

Chemistry 1950

Presentation Speech by Professor A. Fredga, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Royal Highnesses, Ladies and Gentlemen.

With this year's Nobel Prize for Chemistry, the Academy of Sciences wished to signalize the discovery and development of diene synthesis. This achievement falls within the domain of classical organic chemistry – the chemistry of carbon compounds. The field in question is somewhat recondite and not easy to describe in words « that everyone can understand » – a fact to which the scientists whose task it has been to explain the Nobel Prize in the press can testify. The chemistry of carbon compounds is rather like a Chinese puzzle in which the pieces consist of atoms – a puzzle with simple fixed rules and a great many possible combinations. The task of the research scientist is twofold: on the one hand he has to discover how Nature has arranged the puzzle in the thousands of compounds which we meet in the animal and vegetable kingdoms, whilst on the other he has to imitate, modify, and supplement the work of Nature. A fascinating game for anyone who knows the rules!

Diene synthesis occurs between two different molecules of more or less complex structure. One of them, the diene, contains a chain of four carbon atoms, linked by two double bonds and – in the centre of the chain – a single bond. The first syllable of *diene* is the Greek word for « two », and refers to the two double bonds. The other molecule must contain two carbon atoms linked by a double bond, and must also satisfy certain other structural requirements. This component is usually called the dienophil or philodiene, i.e. the diene-loving, partner. In diene synthesis the two chains attach themselves to four or two carbon atoms to form a ring, which thus contains six atoms; this number is particularly favourable to cyclization. At the same time the double bonds are broken, so that only *one* remains in the new system.

It has long been known that molecules with double bonds readily become attached to one another; today we also know that this process usually results in very long atom chains, known as giant molecules. Diene synthesis is a special case, where, owing to certain geometrical and arithmetical relations,

growth of the atom chain by attachment of new molecules ceases. Instead, the chain catches its own tail, so to speak, and forms itself into a ring which can grow no further.

The reaction occurs with astonishing facility, often quite spontaneously, without external heating, addition of condensing agents or the like. It is perhaps surprising that this reaction, which is extremely elegant from the chemical point of view, should not have been discovered earlier, for cyclic structures had in fact been known since the sixties of last century. Individual observations had been made but they had been misunderstood or overlooked. The correct interpretation was so simple and yet – a mere twenty years ago – so bold, so like a chemist's utopian dream, that it was beyond reach.

Diels and Alder were interested in a particular, not entirely simple, form of this reaction, which had formerly been misunderstood, and they gave the correct interpretation of it. They also suspected that they had found a general method of synthesis of great fundamental significance, and their conjectures were confirmed by many investigations in various directions. This achievement was described by a famous French scientist as « profondément intuitive et géniale ».

This was more than twenty years ago, and diene synthesis has now developed into one of the most important working methods in organic chemistry. By means of this method a large number of compounds of complex structure can easily be produced – compounds which it would be quite impossible or very difficult to produce in any other way. An example is the peculiar carbon framework of the camphor molecule. The method has also proved valuable in a great many ways in research into the constitution of complex natural products; these include the resinic acids and cantharidin, the active substance – with very peculiar chemical properties – of the « Spanish fly » (which is actually a beetle).

The compounds present in essential oils and natural resins often possess a diene structure. Reaction with suitable philodienes produces substances with properties valuable to industry, e.g. as raw materials for plastics. Numerous applications for patents testify to this. Among the many industrial applications of diene synthesis one might also mention analysis and refining of synthetic motor fuels.

The products formed by diene synthesis are usually stable, although sometimes they break down into their components at high temperatures. In a few cases, however, disintegration takes a different form – here, a part of the

molecule is said to be «expelled». This results in new substances – in some cases substances which cannot be obtained in any other way.

The many theoretical problems which diene synthesis has created or brought into prominence cannot be discussed here.

Professor Diels, Professor Alder. More than two decades have passed since the day when you sent in the first paper on «Syntheses in the Hydroaromatic Series» to the Editor of the *Annals*. Much has happened since then – in Science, as well as in the world.

The reciprocal addition of the unsaturated compounds was at that time still somewhat mysterious. The chemistry of the high polymer compounds belonged for the most part to the future. You found it necessary to stress that the new substances which you obtained so easily were not molecular compounds but were really new molecules, stable and firmly bonded. New simple bonds had come into being of the old, well-known kind. This identification has paved the way to a right understanding of the polymerization phenomena of the unsaturated compounds, and through it you have very greatly advanced the development of high polymer chemistry and technics.

In this treatise you have even indicated how highly promising the consequences of the low molecular compounds were for the future of chemistry. In the past years the synthesis of dienes has, to be sure, achieved more than you could either foresee or predict when you wrote your paper. But the general trend, the prophetic leading ideas are already to be found in it.

You mention there the possibilities for discussion of several theoretically interesting questions concerning the forces of attraction in polycyclic systems. You also mention the practical consequences. The possibility of the synthetic production of complicated natural substances or of compounds similar to them is, in your view, immediately in prospect. Finally you indicate the possibility that this astonishingly rapid and smoothly running reaction could also play a significant part in the processes of Nature.

Since 1928 the synthesis of dienes has been developed in various directions by numerous workers, and its significance has become more and more obvious. You have yourselves, partly together, partly independently, worked with great success in the field you have opened up. The external circumstances were not always of the best for reasons which we all know. We have been told that you, Professor Diels, were in the past years unable to do any experimental work. And yet your thoughts played keenly upon scientific

problems and we hope that you will soon have the opportunity of approaching the problems experimentally as well. You, Professor Alder, in spite of limited facilities for work, are again fully active. During the past year you have, by your splendid work on the steric and energetic conditions in the polycyclic systems, successfully maintained your position as the leading research worker in this field.

Most esteemed colleagues: in recognition of your services to chemistry through the discovery and development of the synthesis of dienes, the Royal Academy of Sciences has decided to award you the Nobel Prize for this year.

I convey to you the most sincere congratulations of the Academy and ask you to come forward and receive the Nobel Prize from the hands of His Majesty the King.

Otto Diels

Description and importance of the aromatic basic skeleton of the steroids

*Nobel Lecture**

Cholesterol was discovered as long ago as 1789[1]. Since it was found to be widely distributed in both the plant and the animal organism, and since it plays an important part in the latter (being present in blood, brain, liver, egg yolk, gall-stones, etc.), it is understandable that many chemists and physiologists should have directed their efforts, especially during the second half of the 19th century, towards determining the composition and structure of this remarkable and important substance.

Until the beginning of the 20th century, however, the results of these researches were but modest, and the hope of discovering the nature of cholesterol and of its chemical structure had not been realized.

All that had been established was its composition, $C_{27}H_{46}O$, and the fact that the cholesterol molecule contained one double bond and one alcoholic OH group.

This was certainly not very much! Nevertheless, on the basis of these results it had been established that cholesterol was a complex hydroaromatic system composed of four rings. Until 1900, however, all efforts to explain this system, e.g. by oxidative modification and by determination of the character of the cleavage products, had failed.

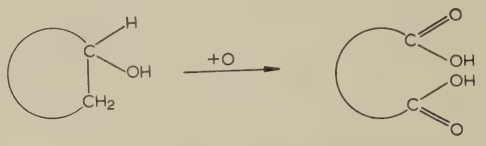
Two years later I myself succeeded in making two important observations on cholesterol. Firstly that with NaOBr it could be transformed fairly easily into a dicarboxylic acid with very good crystallizing properties and with the same number of carbon atoms as cholesterol:

$$C_{27}H_{46}O \xrightarrow{+O} C_{27}H_{44}O_4$$

From this it was quite clear that this dicarboxylic acid was produced as a result of the splitting of one of the four rings of the cholesterol.

* Professor Diels intended to deliver his Nobel Lecture during a visit to Stockholm in May, 1951. He had completed the manuscript of this article but was prevented by illness from coming to Sweden and delivering the lecture.

Secondly, since the dicarboxylic acid no longer contained the OH group of the cholesterol, it was evident that its formation from cholesterol must involve the following process:



There followed from this the further important fact that the OH group was not in a side chain in the cholesterol but was linked to a ring system.

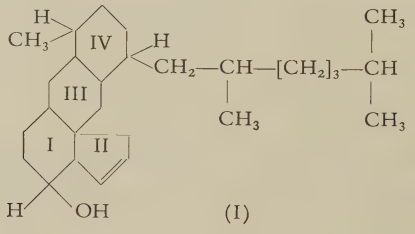
At about the same time I proved that this OH group was a secondary alcohol group, for I succeeded – which had until then been attempted in vain – in converting cholesterol by a very simple method, namely by catalytic dehydration, into the corresponding ketone: cholestenone:

$$C_{27}H_{46}O \xrightarrow{-H_2} C_{27}H_{44}O$$

After these successes I withdrew for the time being from cholesterol research because, owing to the ever-increasing number of collaborators, I had to familiarize myself with many other fields of organic chemistry.

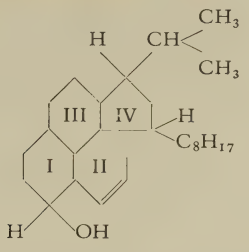
During the next two decades it was primarily Windaus and Wieland who devoted themselves – and with great success – to research on cholesterol. It was a satisfaction to me that the two compounds which I had discovered – the dicarboxylic acid $C_{27}H_{44}O_4$ and the cholestenone $C_{27}H_{44}O$ – played an important part in these investigations and advanced the research in a not inconsiderable degree.

Thus, elucidation of the complex cholesterol molecule proceeded step by step, until already by 1919, Windaus[2] was able to suggest a tentative cholesterol formula (I):



(I)

A few years later, however, Wieland, Schlichting, and Jacobi suggested, on the basis of the results of their research, a different structural formula:



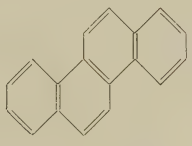
which differs from that of Windaus by its two five-membered rings, as well as in other important respects.

It will be seen, then, that ideas on the true structure of cholesterol, and therefore on its formula, were very vague at that time.

This uncertainty prompted me, remembering my former successes in this field, to try to «get through» to the aromatic basic skeleton and determine its character by dehydration of cholesterol.

I was fully aware, of course, that many unsuccessful attempts to do this had been made with a great variety of dehydrating agents. The problem, therefore, was to discover new dehydrating methods with a specific action.

First, I tried with palladinized charcoal in boiling cholesterol, i.e. at approximately 400°C. I had some success here in so far as I actually obtained an abundant yield of an aromatic hydrocarbon, which could be characterized satisfactorily as the familiar chrysene:



Chrysene

However, for the structural formula – and this was the problem – it was scarcely possible to draw any useful conclusion from this observation, since at the high dehydration temperature rearrangements to the chrysene, which is exceptionally stable, are quite possible. Later, however, I was able to prove that chrysene is directly related to the true basic skeleton of cholesterol.

In other fields of organic chemistry sulphur had proved to be a very useful dehydrating agent, but with cholesterol it had been a failure because its action was too vigorous, and because it entered the molecule by substitution. Thus, all in all, dehydration of cholesterol with sulphur resulted in an amorphous mixture of semi-viscous substances which could not be separated from one another.

Then, fortunately, I hit upon the happy idea of replacing sulphur by selenium, because the action of this element is much more gentle than that of sulphur, and because it does not have the tendency to enter organic molecules.

This new dehydrating agent was an unexpected success.

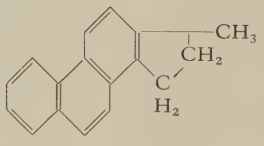
By heating melted cholesteryl chloride or cholesterol with selenium powder until evolution of hydrogen selenide had ceased, and by fractionating the reaction product I obtained, as main product, a large yield of a beautifully crystallized aromatic hydrocarbon with the composition $C_{18}H_{16}$ and melting point 124–125°C. This hydrocarbon has loomed very large in the literature on organic chemistry during the past two or three decades.

Since this wholly unexpected result, obtained by a method – «selenium dehydration» – which I had recently discovered, aroused much interest everywhere, it was only natural that, like a great many chemists throughout the world, I should have attempted to explain the structure of this hydrocarbon $C_{18}H_{16}$, which was obviously the aromatic basic skeleton of cholesterol. Strangely enough, however, the task was by no means easy, despite the fact that this was an aromatic system.

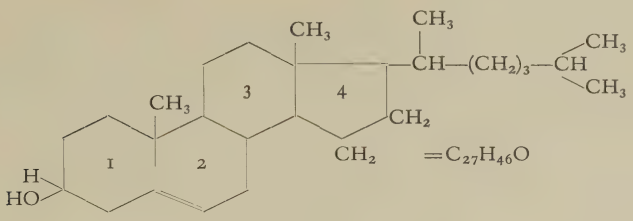
And so it was that just before my own investigation had been brought to a successful and decisive conclusion, Harper, Kon, and Ruzicka[3] forestalled me by establishing the identity of the γ-methylcyclopentenophenanthrene, which they had produced by synthesis, with the hydrocarbon $C_{18}H_{16}$ which I had obtained by dehydrating cholesterol with selenium.

Shortly after this I myself again proved the identity of the two products by converting them into the same tribromide[4].

Subsequently the composition and correctness of the formulation of the important hydrocarbon $C_{18}H_{16}$ was checked and confirmed in many different quarters, and consequently the following structure has been definitely assigned to it:

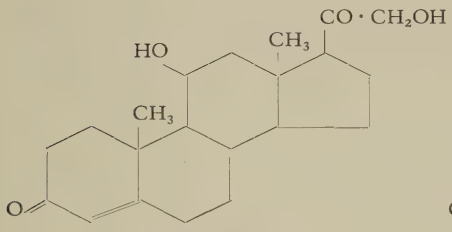


The first tangible result of this discovery was the certainty that a structural formula agreeing with all experimental observations could now be drawn up for cholesterol:

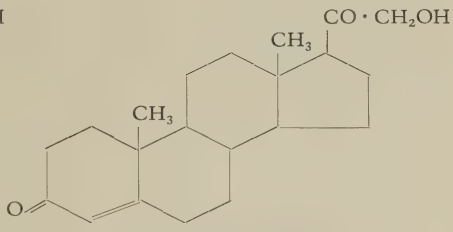


Much more important for science, however, was the fact, which was gradually emerging, that an intimate connection exists between cholesterol and a large number of substances of very great importance in the medical, physiological, and biological fields. Of these we will mention in particular ergosterol and the other steroids, bile acids, sex hormones, the « heart genins » (digitoxigenin, gitoxigenin, strophantidin, peristogenin, uzarigenin, etc.) and the neutral sapogenins.

The hormones corticosterone and desoxy-corticosterone:



Corticosterone

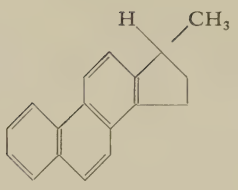


Desoxy-corticosterone

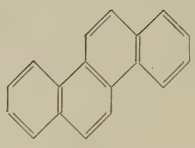
which have very recently been isolated from suprarenal cortex by Kendall and Reichstein likewise contain the characteristic ring skeleton of γ-methylcyclopentenophenanthrene, as well as all the other above-mentioned natural substances in hydrated form.

All these compounds and many others can be transformed into the hydrocarbon $C_{18}H_{16}$ with its reliably determined structure of γ-methylcyclopentenophenanthrene by the method which I discovered for dehydrating cholesterol and which I introduced into science as « selenium dehydration ».

Even the remarkable observation which I described a little earlier on, namely the formation of chrysene during severe dehydration of cholesterol with palladinized charcoal, is now explainable, for it is understandable that γ-methylcyclopentenophenanthrene (I), which is probably the first substance to be produced by dehydration:



(I)



(II)

should be transformed into chrysene (II) – which is extremely stable – under the much more vigorous conditions of dehydration with palladinized charcoal at raised temperature, and that the process should be accompanied by ring extension and splitting-off of hydrogen.

It will rightly be asked: What is the synthetic principle on which this obviously highly important product of γ-methylcyclopentenophenanthrene is built up in nature, and why is it that this particular type which, as foundation of many substances indispensable to life and of extreme physiological and biological importance, plays such a vital role in the vegetable and animal kingdoms? However, the time has not yet come when we can give an answer to questions so fundamental and so important to an understanding of the workings of Nature. But I am firmly convinced that this problem – like all others – will eventually be solved.

1. Chevreul, *Ann. Chim.*, 1 (1789) 95.
2. A. Windaus, *Nachr. Kgl. Ges. Wiss. Göttingen*, (1919) 237; *Chem. Ber.*, 52 (1919) 162.
3. S. H. Harper, G. A. R. Kon, and F. C. J. Ruzicka, *J. Chem. Soc.*, (1934) 124.
4. O. Diels and F. Rickert, *Chem. Ber.*, 68 (1935) 267.

Biography

Otto Paul Hermann Diels was born in Hamburg, Germany, on January 23, 1876. When he was two years of age the family moved to Berlin, where his father was appointed to a professorship. His early education, from 1882 to 1895, was at the Joachimsthalsches Gymnasium, Berlin. In 1895 he went to Berlin University where he studied chemistry, together with other science subjects, under Emil Fischer, graduating in 1899. He was at once appointed assistant at the Institute of Chemistry at Berlin University, becoming a lecturer in 1904. Promotion to Professor followed in 1906, and he was appointed Head of Department in 1913. He became Professor at the University of Berlin in 1915 but, the following year, moved to the University of Kiel as Professor and Director of the Institute of Chemistry. There he remained until his retirement in 1945.

His earliest research was in the field of inorganic chemistry, where he was the discoverer of an oxide of carbon having some unusual properties – carbon suboxide. His subsequent work was in the domain of organic chemistry. He was responsible for introducing the use of selenium as a specific reagent for the dehydrogenation of hydroaromatic compounds (1927). This proved to be a very useful tool in elucidating chemical structures in the complicated steroid series, where his name is associated with the hydrocarbon 3′-methyl-1,2-cyclopentenophenanthrene. Diels obtained this skeletal steroid structure by dehydrogenating cholesterol, and other members of the series, with selenium.

His best known work was done in collaboration with Kurt Alder on the chemical reaction which bears their joint names (1928). This, sometimes known also as the diene synthesis, consists in the reaction of a diene with a second component which has carbonyl or carboxyl groups adjoining an ethylenic bond, to give unsaturated cyclic compounds. As the two reactants can be varied widely within the scope of this definition, a very large range of new compounds can be prepared. The reaction takes place without the need for forcing conditions and, by its use, many complicated natural products, and modifications thereof, may be synthesized.

Diels was the author of the *Einführung in die organische Chemie* (1907), which went through fifteen editions. He had many papers published, mostly in German scientific periodicals such as *Liebigs Annalen der Chemie*.

One of his earliest awards was in 1904, when he was awarded a Gold Medal at the St. Louis (U.S.A.) International Exhibition. He won the Adolf v. Baeyer Medallion in 1930 and the Grosskreuz des Verdienstordens der Bundesrepublik Deutschland in 1952. He held the honorary degree of Doctor of Medicine from the University of Kiel, and was a member of the Academies of Halle, Munich, and Göttingen.

Diels married, in 1909, Paula Geyer. They had three sons and two daughters; two of their sons were killed in action during World War II. He was interested in reading, music, and travel for his recreation, and, in his younger days, he had been fond of mountaineering. He died on March 7, 1954.

Kurt Alder

Diene synthesis and related reaction types

Nobel Lecture, December 12, 1950

The Precursors

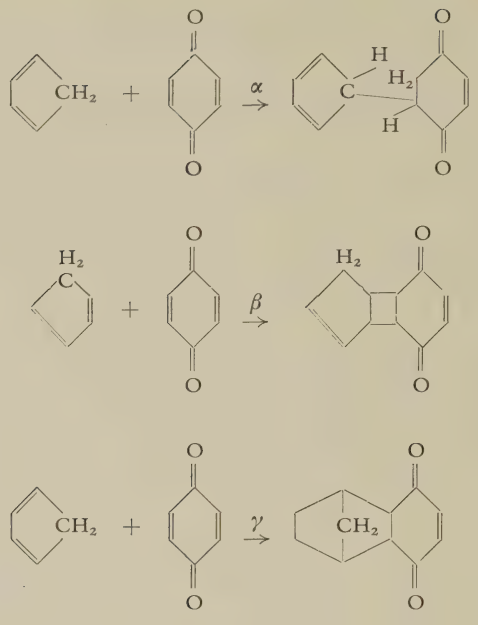
Since the Nobel Foundation was first established, the Royal Swedish Academy of Sciences has deemed two advances in the field of organic chemical synthesis worthy of distinction by the award of a Nobel Prize. These were V. Grignard's discovery of the organomagnesium compounds and their application for preparative purposes, and P. Sabatier's discovery of catalytic hydrogenation, i.e. the addition of elementary hydrogen to unsaturated compounds by means of contact catalysts. Both methods were very soon introduced into all laboratories, where they became indispensable for the everyday preparative work (which would now be unthinkable without them), and both – especially the latter – have been introduced, in progressively improved form, ever more widely into industrial technology.

There has also been a third advance in the preparative field, namely diene synthesis, which is now a routine working method in organic chemistry. And it is for the discovery and development of this process that my colleague Diels and I have this year received the highest honour in the form of the Nobel Prize for Chemistry.

Two substances and their behaviour seem to me to be particularly suited for a description of the situation which served as the point of departure for the developments initiated by Diels and myself in 1927, namely cyclopentadiene and isoprene.

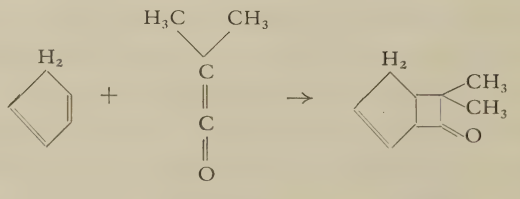
Cyclopentadiene (I) is characterized, as far as its reactive properties are concerned, by two factors: the presence of two (conjugated) double bonds, and the mobility of its hydrogen atoms in the methylene group. This theory is based on general experience, gained at an early date, in the conversion of this substance with agents such as bromine and alkali metals. When subsequently (1906) it was discovered that the hydrocarbon derivative quinone undergoes addition with extreme facility, the most obvious thing was to attribute this process to the same factors and to discuss the following possibilities (α–γ).

(I)



The formula (α) first recommended by its discoverer[1] is based on the assumption that a methylenic hydrogen atom is displaced from the cyclopentadiene range to the quinone range, whereas the subsequently suggested interpretations β and γ assume the interplay between the double bonds of the two partners to be – each in its own way – a case of straightforward addition. As there was no proof of the constitution of the addition product, there was equal justification for all the hypotheses so long as they rested only on unreliable analogies.

True, in the light of a further observation on cyclopentadiene the choice appeared temporarily to lie between reactions α and γ. The mutual formation of two double bonds and the formation of a four-membered carbon ring (III) was proved in the case of an addition product of dimethylketene (II) with cyclopentadiene[2]:

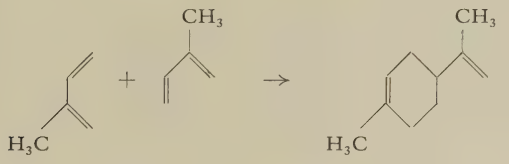


(II) (III)

The parallel between these two processes seems obvious, but in fact does not hold. Only very much later was it shown that this reaction is very largely confined to ketenes and that it cannot be carried over to the addition of quinone to cyclopentadiene or to the addition of cyclopentadiene to similar substances, i.e. to the polymerization of cyclopentadiene.

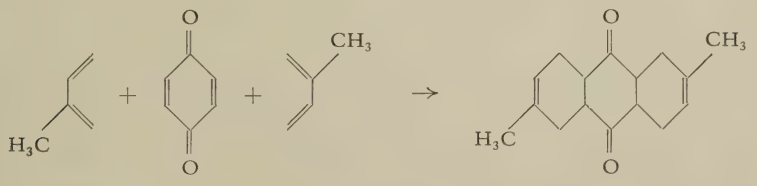
This discovery was, however, the fruit of later work. For the time being the addition of ketene seemed quite obvious as a paradigm for the other additions accompanied by the formation of C–C bonds. Indeed, even at that time, other analogies showed that a reaction in accordance with formula γ was possible, in particular the addition of azodicarboxylic ester to cyclopentadiene, which can be proved to take place in accordance with formula γ although it is accompanied by formation not of C–C but of C–N bonds[3].

Two observations on isoprene (IV) pointed in the same direction. The classical dipentene synthesis (V) dating from 1885[4] requires relatively high temperatures and gives poor yields. This may account for the fact that for decades it was regarded as an exceptional case.



(IV) (V)

It was not until 1920[5] that as a second contribution of this kind the addition of two molecules of isoprene to quinone – in a very smooth reaction – was described and the process was given the substantially correct although as yet unproved formula[6].

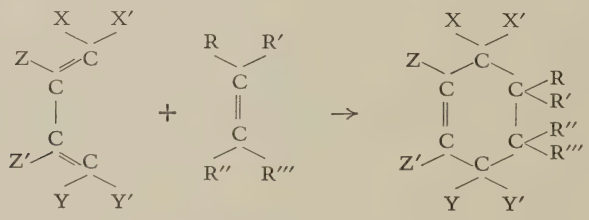


In view of this situation – three types of reaction side by side, with an indefinite boundary between them – a need for classification and systematization was inevitably felt. There was only one certain way of achieving this objective, namely by establishing the constitution of the addition products by direct proofs. Today, now that we have highly developed methods of

proof and plenty of reliable cases to draw on, it is difficult to imagine that at that time it was the search for provable cases which necessitated a variation of the two addenda – cyclopentadiene or isoprene on the one hand and quinone on the other. The shift of interest to the prototype of the dienes, butadiene, and the important step from quinone to maleic acid, which was later followed by those to acrylic acid and acrolein, led so inevitably to the goal[7] that our principle of a successive structural modification of the components remained the «leitmotiv» of our joint work. Subsequently, contributions on the same subject, though occasioned by the widest variety of causes, have been made on a rapidly increasing scale by many other authors, and have gradually built up a complete picture of these reactions.

The General Formula

The feature common to all these processes is the formation of a six-membered carbon ring by a direct union, i.e. a union without adjuvant substances, of two unsaturated partners. One of these, the diene component, is the carrier of a system of conjugated double bonds whilst the other, the «philodiene», must have at least one double bond. The addition usually takes place in accordance with the following scheme:



Diene Philodiene Addition product

This basically uniform picture acquires its undreamed-of wealth of variations of all kinds thanks to the individual peculiarities of the reaction partner, in other words: thanks to the different substituents. These substituents are variables which, with the well-known profusion of forms of organic molecules, are available in true abundance.

From the great variety of these possibilities a large number have been realized by very many different authors in the course of over twenty years' development. Their results can be summarized by saying that in the overwhelming majority of cases they follow this basic formula, irrespective of

whether they belong to the ideal cases which proceed with absolute ease or whether they require high temperatures, and irrespective of whether their addition products are stable or sensitive to heat. For them the 6-ring formation is undoubtedly a law.

The diene component

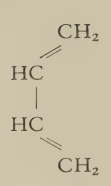
In this lecture I shall not dwell on the normal cases, for I can scarcely take up your time with matters which today are learnt by any beginner and have to a large extent been tested and proved and therefore no longer have the attraction of novelty.

However, in order to give at least a very rough idea of the scope of the basic formula and of the nature and multiplicity of the substituents, I have summarized in two tables the primary types of the cases hitherto investigated. In these tables, wherever a representative of the one reacts with a representative of the other, this reaction is in accordance with the formula of diene synthesis.

The range of validity of the basic formula is very wide; it extends from the simplest case of butadiene and of the acyclic dienes (VI) to the alicyclic combinations, in which both double bonds are situated in one ring (VII), or are spread over a ring and a side-chain (VIII) or two rings (IX). In addition to this, various empirical findings have shown that the basic formula does not lose its validity even if, as in the case of styrene (X), methylene anthrone (XI) or other «semi-aromatics», one of the double bonds of the conjugated system belongs to an aromatic nucleus or if, as in the case of the anthracene type (XIV), both double bonds belong to an aromatic system. No diene syntheses have so far been observed with mononuclear benzene derivatives (XII), and according to very recent findings[8] they do not occur in the case of binuclear derivatives of the naphthalene type, unless at least one of the two nuclei is highly alkylated. Heterocyclic dienes of the furan type (XVI) and of the coumalin type (XV) give diene synthesis, but simple derivatives of thiophen (XVII) do not. We shall be discussing the behaviour of pyrrole (XVIII) later on (p. 277).

Acyclic tri- and tetraenes – in the simplest case hexatriene (XIX) – show normal behaviour with diene synthesis, i.e. they too add philodienes in accordance with the formula of the 6-ring formation. The predictable formation of higher-membered rings by 1,6- or 1,8-addition has not yet been confirmed empirically, at least not with acyclic tri- or polyenes, on which

Table of dienes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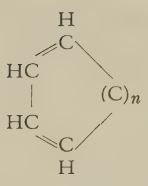
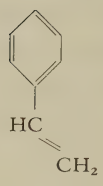

(VII)

(VIII)

(IX)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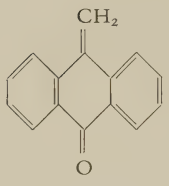
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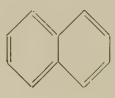
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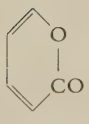
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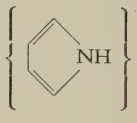
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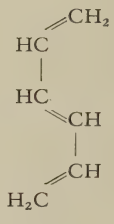
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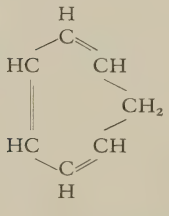
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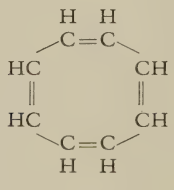

(XXI)

extensive experimental data[9,10] are available. Their cyclic analogues such as cycloheptatriene (XX)[11] and cyclooctatetraene (XXI)[12] are likewise capable of diene synthesis – indeed, to a very impressive extent – thanks to the nature of their double-bond systems.

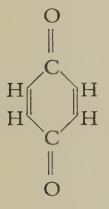
The philodiene partner

The basic formula of addition characterizes the philodiene as a derivative of ethylene. Precisely in this group, extensive experience has shown that not

every olefine bond adds equally rapidly, but that the absorption capacity of the bonds depends to a large extent on constitutional conditions.

The α,β-unsaturated carbonyl compounds (XXII–XXX) are at the head of the entire group. They provide optimum conditions for adaptation to diene synthesis. Recognition of this fact, especially in the case of the *p*-quinone series (XXII), maleic anhydride (XXIII), acrylic acid (XXIV) and acrolein (XXV), constituted the decisive step in the discovery of the new method[7]. Subsequently these compounds also played a decisive part in the efforts to arrive at a thorough understanding of diene synthesis, and work on the adaptation of it to industrial purposes was, and still is, based primarily on them.

Table of philodienes



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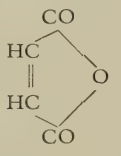

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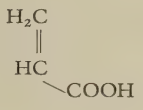

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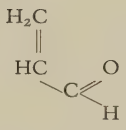

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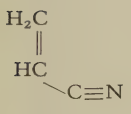

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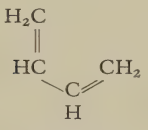

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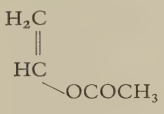

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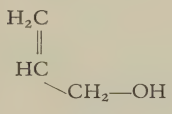

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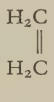

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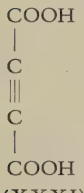

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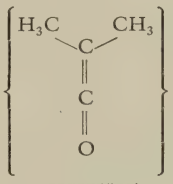


(XXXIII)

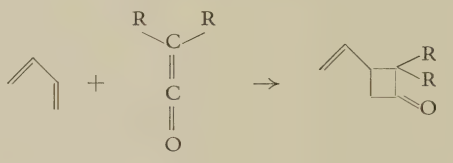
I must confine myself here to a brief survey of the philodiene class, with a rapid glance at its principal representatives, without attempting to go into any details.

Beside the α,β-unsaturated carbonyl compounds there are also the nitriles of α,β-unsaturated carboxylic acids (XXVI). The great tendency of many dienes to dimerize in accordance with the formula of the « partial 1,4-addi-

tion» explains why the simple dienes C=C—C=C (XXVII) can also act as philodienes.

Vinyl compounds (XXVIII), such as are now being produced industrially on a large scale for other purposes from acetylene react like allyl compounds (XXIX) with dienes in accordance with the diene synthesis formula; the same applies to hydrocarbons such as styrene, indene, propylene, and the simplest structure of this type, ethylene (XXX) – last but certainly not least of the series of philodiene components.

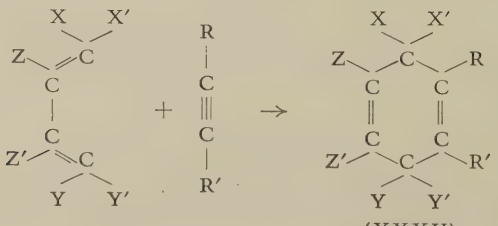
Within this wide range of olefine compounds the ketenes must be specially mentioned as the only exception so far known. They add dienes with the mutual elimination of one double bond each and with formation of derivatives of cyclobutane (XXXIV):



(XXXIV)

This rare case, which is undoubtedly due to the characteristic accumulation of double bonds in the ketene system, was first observed and defined long before diene synthesis was recognized as a general principle. The formation of four-membered rings from ketene and dienes has recently been confirmed many times over[13]. It is clearly an addition form typical of the ketenes, or in other words the ketenes cannot act as philodienes in the above-defined sense. Whether and to what extent this rule also applies to the allenes $H_2C{=}C{=}CH_2$, which are closely related to the ketenes, is a question which still remains to be answered.

On the other hand, the derivatives of acetylene also fit without limitation into the general formula for diene synthesis:



(XXXV)

Acetylene dicarboxylic acid (XXXI), propiolic acid (XXXII), and other acetylene derivatives have proved themselves to be very productive philodienes. Their doubly unsaturated addition products (XXXV) constitute a supplement – for many purposes ideal – to the addition products of the olefine series.

The role of maleic and fumaric acid

The results obtained from and the problem raised by diene synthesis from the point of view of general methodology are the subject of my lecture. I shall therefore not go into details on the many applications – which in course of time this method has made possible – for the analysis and determination of the constitution of organic compounds, and for preparative and industrial purposes. Instead, allow me here to make a brief observation.

I have already implied that, with regard to method, the step from quinone to maleic anhydride was of decisive importance in the development of diene synthesis. The propensity of maleic anhydride, and with it of related substances, to act as philodienes, which this revealed, is so complete that it could not escape our attention. The suspicion that this was the case was subsequently confirmed, and indeed is continually being strengthened as experience grows, and I should scarcely be going too far if I said that the suitability of substances of this type for preparative chemistry was discovered only with diene synthesis – almost exactly a hundred years after maleic acid and its close relative fumaric acid had first been isolated from plant material. Several decades after their discovery they became the focus of theoretical discussions, when the ideas of Van 't Hoff and Le Bel on the arrangement of the atoms in space threw the first light on their hitherto problematic relations to one another. Having been recognized as examples of *cis*- and *trans*-isomeric forms, and accepted as classical types, they ceased to be of immediate interest, until some decades later, fumaric acid – after being found by Thunberg and Wieland to be a transitional product in the metabolism of carbohydrates – attracted the attention of physiologists.

Despite this importance with regard to theoretical and biochemical problems, maleic and fumaric acid remained laboratory and academic preparations, which were more or less difficult to produce, and provided scarcely any greater incentive to efforts in the field of synthetic organic chemistry than any other olefine.

In the history of chemistry there can be few similar cases where sub-

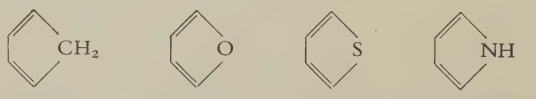
stances which during a hundred years' existence were repeatedly in the forefront of scientific development and then suddenly revealed a latent capacity and – so to speak, overnight – attracted the interest not only of a few specialists but of a wide range of workers in pure and applied organic chemistry. Thus, today it is difficult to find a type of unsaturated compound whose reaction with maleic anhydride has not been investigated, at least provisionally. The interest shown by industry in this development is reflected in the number of applications for patents based on combinations of maleic and fumaric acid, their homologues and functional derivatives, with an extremely wide variety of unsaturated compounds. Present efforts to develop inexpensive methods of producing maleic and fumaric acid from substances such as benzene, crotonaldehyde, and furfural originated in the needs which have arisen from the new applications of these substances.

After this diversion I should like to turn my attention to the nature of the reaction. Here, too, the importance of maleic and fumaric acid will repeatedly find expression – even in the extreme case of pyrrole, which I shall now discuss.

Substituting Addition

Substituting addition to the double bond

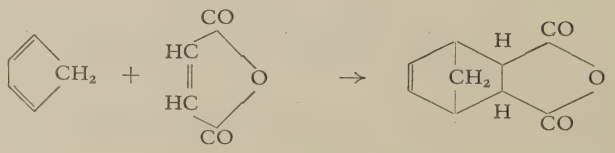
One of our series of investigations was concerned with testing the reactions of the systems



(XXXVI) (XXXVII) (XXXVIII) (XXXIX)

with maleic anhydride. The similarity is obvious: true pentacyclic systems, all characterized by the presence of a pair of conjugated double bonds, the first belonging to the isocyclic, the rest to the heterocyclic series.

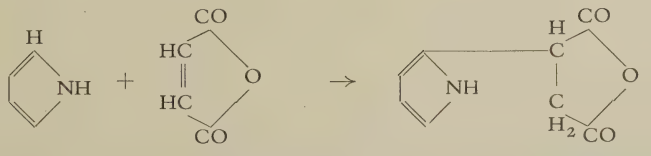
It had already been established that cyclopentadiene (XXXVI) adds maleic anhydride with unusual facility and that this process constitutes the ideal case of diene synthesis.



Furan (XXXVII), on the other hand, showed only differences of degree. The product of addition to maleic anhydride was less stable, and a determination of its constitution left no doubt that in its formation the same principle was at work as in the case of cyclopentadiene.

Thiophen (XXXVIII) under the same external conditions does not react at all with maleic anhydride, a result which it has so far been impossible to alter even by forcing the experimental conditions. However, thiophen may not be such an exceptional case as this might suggest. It is well known that in thiophen interaction between the double bonds and the sulphur atom brings about an internal equilibrium which can be compared with that of benzene and which expresses itself in a startling similarity in chemical and physical behaviour between the two substances. Benzene is likewise incapable of diene synthesis. But in the same way as the benzene system assumes the character of a diene with more highly condensed types, i.e. it acquires a capacity for attaching maleic anhydride in the 1,4 position, so the arrangement of the thiophen, when embedded in relatively complex systems, attains the same capacity for true diene synthesis.

The situation with pyrrole (XXXIX) is different[14]. This substance reacts very readily with maleic anhydride. These additions, however, are not diene syntheses, but rather result in the setting-up of only one double bond while at the same time displacing a hydrogen atom from the range of the one component to that of the other. According to their products, these, like diene syntheses, are additions; according to their nature they are undoubtedly substitution processes. I have therefore suggested for them the name «substituting addition»[15].



It was subsequently found that maleic anhydride reacts in accordance with the formula of substituting addition not only with pyrrole but also with many other nitrogen-containing heterocyclics, such as pyrazole, glyoxaline, indole, etc. The study of this vast subject is not yet complete, and we shall not be examining it in any further detail here. The main result lies in the proof that maleic anhydride is not only an ideal philodiene in diene synthesis but is also particularly suitable as a hydrogen acceptor in substituting additions.

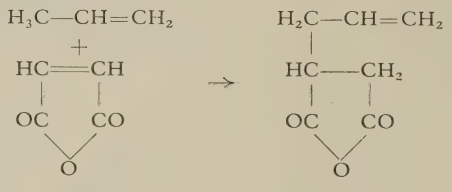
We describe this category of substituting addition – namely where a hydrogen atom of the group C=CH is detached – as «substituting addition at the double bond», thus distinguishing it from processes of a similar kind, which will now be discussed.

Substituting addition in the allyl position

The beginnings of this development were prompted by statements in the patent literature, according to which maleic anhydride can be added even to singly unsaturated hydrocarbons at 180–250°C[16]. This gives rise to addition products which contain the components in the molar ratio of 1:1, and which at first were all classified as «substituted succinic acids».

For an understanding of the phenomenon of substituting addition we considered it important that these statements should be supplemented by systematic experiments and that the constitution of the addition products should be determined for certain.

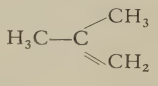
We established that ethylene itself is not capable of forming a simple addition product with maleic anhydride, at least not under the same conditions as those under which other olefines are transformed more or less easily. The next higher homologue, propylene (XL), reacts with maleic anhydride and was found to give allyl succinic acid (XLI) as its typical and only reaction product.

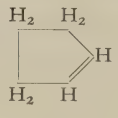


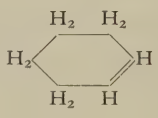
(XL) (XLI)

Analogous behaviour was shown by the following olefines:

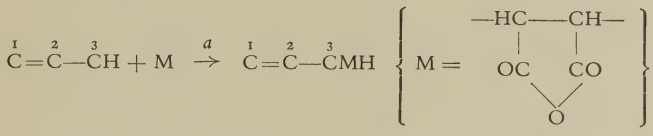
n-Pentene-(2) $H_3C—CH_2—CH=CH—CH_3$

Isobutylene 

Cyclopentene 

Cyclohexene 

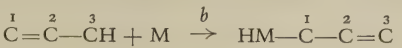
As a result of these experiments, therefore, we found that the additions of maleic anhydride in these cases were the same as the additions to pyrrole and other nitrogen-containing heterocyclics in so far as, like them, they constituted «substituting additions», but that they took place not at a carbon atom of the double bond but at the carbon atom adjacent to it, i.e. in the allyl position.



Thus, the processes of this class seemed to obey a general rule, namely that hydrogen atoms in the vicinity of the double bond are «loosened», and that in the case of substitution reactions – e.g. as a result of the action of Cl_2, O_2 and SeO_2 – they usually tend to be eliminated.

And yet this interpretation of our experiments on the action of maleic anhydride on mono-olefines is incorrect in one vital respect – a conclusion to which extension of our experimental work gradually led us. I will anticipate the result in order to avoid unnecessary digressions.

Our experimental results, which are summarized above, are also susceptible of a different interpretation with the same degree of probability: substituting addition of maleic anhydride to the olefine need not necessarily take place at C_3, but may also take place at C_1, provided that in this case the double bond «shifts» from the C_1, C_2 into the C_2, C_3 position. In this case, too, the hydrogen is detached from C_3:



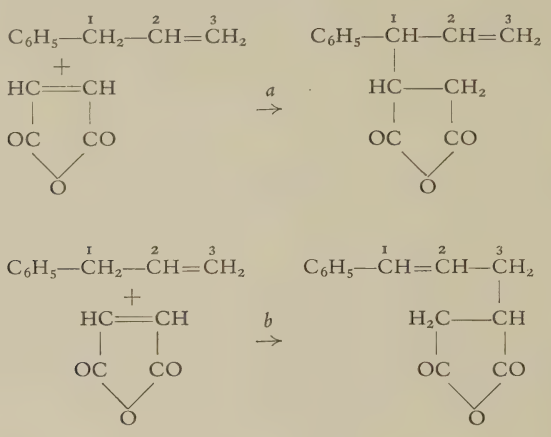
There is scarcely any need to point out that in the cases so far mentioned both assumptions, which we distinguish as *direct* (*a*) and *indirect* (*b*) *substituting addition*[17], are equally justified. This applies in general to all olefines, in which the «shift» of the double bond leads to a substance identical with the original:

Differentiation is possible only where this condition is no longer fulfilled.

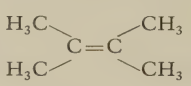
We came upon the first case of this type with allylbenzene (XLII). Here the structures to be expected in accordance with *a* or *b* are different from

one another. An investigation shows clearly that the reaction proceeds in accordance with formula *b*.

(XLII)



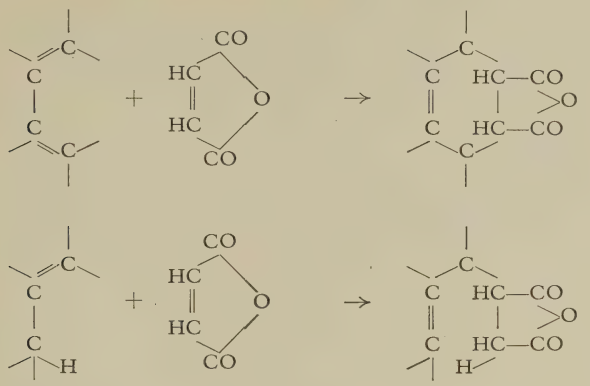
We transferred this finding to other olefines of the allylbenzene type, e.g.

Diallyl	$H_2C{=}CH{-}CH_2{-}CH_2{-}CH{=}CH_2$
n-Hexene-1	$H_2C{=}CH{-}CH_2{-}CH_2{-}CH_2{-}CH_3$
4-Phenylbutene-1	$H_2C{=}CH{-}CH_2{-}CH{-}C_6H_5$
4-Phenylbutene-2	$H_3C{-}CH{=}CH{-}CH_2{-}C_6H_5$
Tetramethylethylene	

In all these cases the process of «indirect substituting addition», which is accompanied by «shifting» of the double bond, always occurs, even where it cannot, for formal reasons, be distinguished from the alternative of a «direct substituting addition», at least in so far as additions of maleic anhydride are concerned[17].

This finding opens up important prospects for our subject and for its future development. It enables the two processes of diene synthesis and substituting addition, which appear to be essentially different, to be examined from the same viewpoint. Diene synthesis is an ideal addition process based on interaction between the double-bond systems of two partners. In view of the migration of the double bond in the substituting addition processes, which has regularly been observed, it can be concluded that these processes are based on a similar effect and that here the group C=C—C—H assumes the function of the conjugated system C=C—C=C in diene synthesis.

A comparison of the two processes:



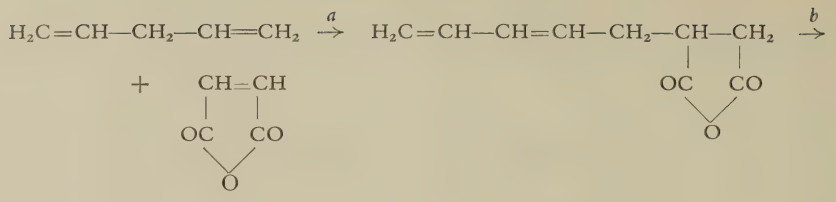
brings out their similarity: in both cases the double bond remaining after the addition process occupies the same position, and in both cases three bonds, i.e. six electrons, change their function. Instead of cyclization, as in diene synthesis, dislocation of the hydrogen atom occurs in substituting addition. It is well known that the dualism (addition–substitution) has always aroused the interest of those whose aim it has been to develop organic chemical methods and to improve and perfect means of describing them. A first attempt to deal with this dualism was made by Kekulé, who put forward the theory that every substitution is preceded by an addition. I cannot help thinking that the substituting addition of maleic anhydride is better suited than that of the substances previously studied to fit in with this view and to bring us closer to an understanding of the nature of substitution.

Combination of Diene Synthesis and Substituting Addition

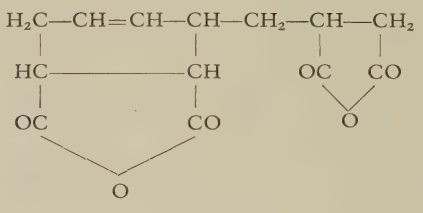
In view of the existence of two definite forms of reaction of maleic anhydride it was possible to foresee cases where both would occur jointly with one another. Two of these seem to me worth special mention – divinylmethane and styrene.

The divinylmethane type

In the first case (XLIII) the maleic anhydride reaction begins, as might be expected, with a substituting addition (*a*).



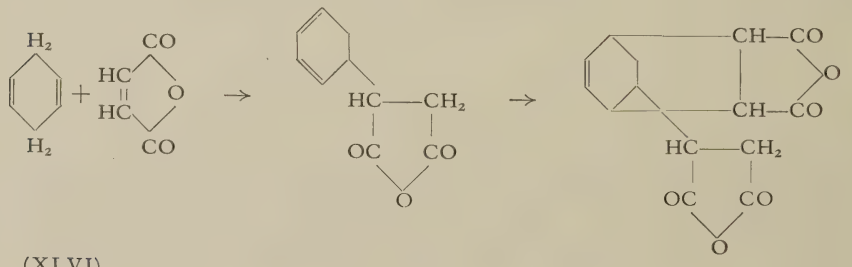
(XLIII) (XLIV)



(XLV)

The «shift» of the double bond connected with this, leads to the formation of a conjugation and thus creates the condition necessary for subsequent diene synthesis (*b*). Since the first reaction is slower than the second, it is impossible to isolate the primary product (XLIV). The result[18] of this transformation sequence is the addition product (XLV), which constitutes a combination of acyclic and cyclic succinic acid. The dianhydride (XLV) – as we have now found – is for its part capable of further characteristic intramolecular changes, a discussion of which would be out of place here.

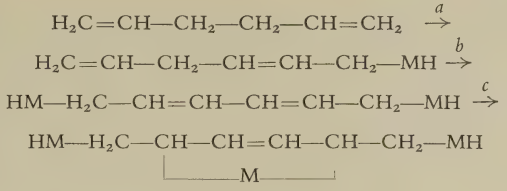
As prototype of the cyclic divinylmethane types we also investigated 1,4-dihydrobenzene (XLVI) from the same viewpoints, and with the same results[18].



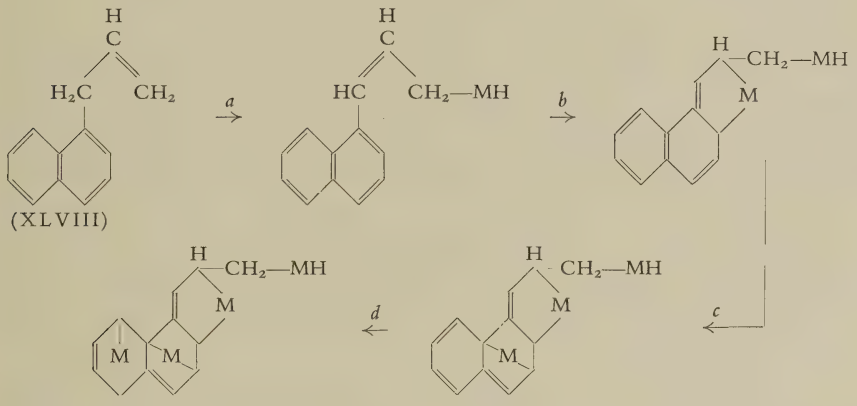
(XLVI)

The coupling of substituting addition and diene synthesis can, with a donor of given constitution, take place on a still larger scale. Thus, diallyl (XLVII) can accumulate up to three, α-allylnaphthalene (XLVIII) as many as four, molecules of maleic anhydride:

(XLVII)



In the first case there are successively two substituting additions (*a* and *b*) and one diene synthesis (*c*); in the second, one substituting addition (*a*) and three diene syntheses (*b*, *c*, *d*).



(XLVIII)

The successive dissolution of the bond system in the naphthalene of the side-chain is particularly significant here[19].

The reaction of the simple divinylmethane types with maleic anhydride also provides the key to an understanding of chemical processes which had hitherto been carried out on account of their industrial importance[20] without so far having led to defined chemical products; I refer here to the effects of maleic anhydride on linseed oil and other oils of the same type. Their common feature is their drying property, i.e. their ability, when in a thin layer, to form a film under the influence of the oxygen of the air. As is well known, this property is dependent on the linoleic (XLIX) and linolenic acid (L) content of these oils.

$$H_3C-(CH_2)_4-[CH=CH-CH_2-CH=CH]-(CH_2)_7-COOH$$

(XLIX)

$$H_3C-CH_2-[CH=CH-CH_2-CH=CH-CH_2-CH=CH]-(CH_2)_7-COOH$$

(L)

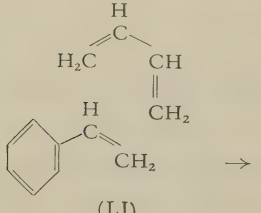
A glance at their structural formulae shows that these, too, are derivatives of divinylmethane. There can therefore be scarcely any doubt about the nature of their reaction with maleic anhydride. In its basic features it will very probably be the same as we found it in the simplest type of this reaction.

The fact that thanks to maleic anhydride these new modifications of drying oils have acquired, and are about to acquire, industrial importance is perhaps of less interest here than the fact that the reaction of divinylmethane with maleic anhydride tells us a great deal about the drying process itself.

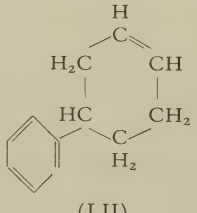
The styrene type

Diene syntheses can be seriously affected if the diene or philodiene component or even both tend to change into pure or mixed macromolecular polymers. Unlike diene syntheses, these processes are chain reactions and are therefore easily influenced by catalysts. Suitable activators trigger them off, effective inhibitors will restrain or even completely suppress them. The boundaries between this kind of process and diene synthesis can be seen very clearly from the example of styrene, and indeed not only in systems where the styrene assumes the function of the philodiene but also in those where it fulfils the role of diene.

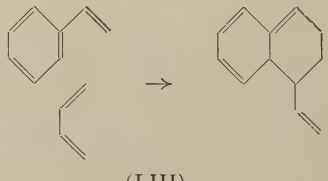
It is well known that the system styrene (LI) and butadiene is changed into macromolecular compounds under the influence of peroxide catalysts, these compounds being the basis of a valuable industrial rubber, «Buna S». There is no need for us to examine these complicated processes in detail in order to show how they differ from diene synthesis – both in the structural formula of the reaction products and in the dynamics of the reaction process. Being a typical chain reaction, polymerization can easily be suppressed, by the addition of inhibitors, to a point where the system can find its spontaneous equilibrium, i.e. the equilibrium no longer forced by catalysts. The diene synthesis which then occurs, results in a partially hydrogenated diphenyl (LII):



(LI)



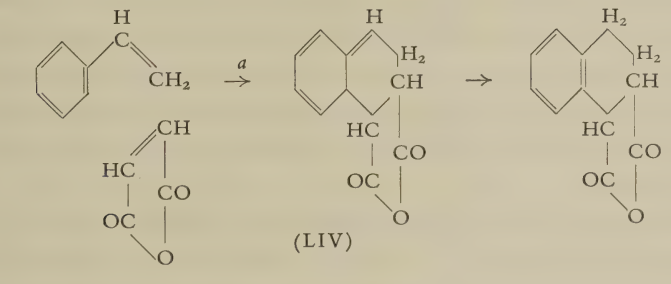
(LII)



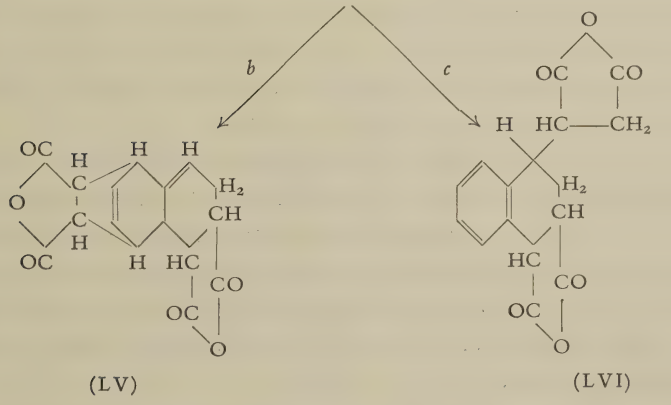
(LIII)

The formation of a naphthalene derivative (LIII) by exchange of the functions of diene and philodiene has so far not been observed, although it is conceivable. The only way to test whether styrene is fundamentally capable of acting as diene was to compare its behaviour with that of a typical philodiene. For this reason the «styrene = maleic anhydride» system was of outstanding interest.

Wagner-Jauregg's first investigations[21], which he carried out as long ago as 1930, showed that here again – as when styrene is combined with butadiene – the picture is completely dominated by the tendency to change into macromolecular «heteropolymers» of linear structure. This tendency was found to be so pronounced here that until very recently nothing could be done about it with the usual inhibitors. It was not until, among the many polymerization inhibitors, the one suitable for our case was found that we were able[19] to study the course of pure unassisted addition in the «styrene = maleic anhydride» system. The result is summarized in the following formulae:



(LIV)



(LV) (LVI)

The first phase (*a*) rests on a 1,4 addition of the maleic anhydride to the mixed aliphatic-aromatic conjugated system of double bonds in the hydro-

carbon, a process during which the bond structure of the phenyl nucleus is broken. There are two possible ways in which the unstable primary product (LIV) can become stabilized. One of these (*b*) is distinguished by the fact that the disintegration of the benzene formation which has been initiated is continued by a second diene synthesis with maleic anhydride. We obtained here for the first time a basic type of an addition product (LV), many variants of which had long been known thanks to the work of other authors[22]. These were obtained from styrene derivatives, in which the tendency to polymerize is not so pronounced as in styrene itself.

A second process (*c*) whereby the primary product (LIV) is stabilized is based on its tendency towards reclosure of the ring. Ideally this takes place by attachment of a second molecule of maleic anhydride in accordance with the formula of «indirect substituting addition». The characteristic «shift» of the double bond and the formation of (LVI), together result in restoration of the aromatic state.

In the case of styrene the second alternative (*c*) occurs more readily than the first (*b*), whereas with α-phenylstyrene the first process is demonstrably the only one which occurs. With substituting styrenes all conceivable intermediate forms between these two processes exist. A study of the connection between the rate at which the two processes occur and the nature and position of the substituents should help towards a thorough understanding of the relationships here.

A detailed examination of the arguments on which our proof is based would be outside the scope of this survey. Mention of the styrenes reminds me that, owing to the imperfections of the methods of organic chemistry, the study of these substances has been a question of patient seeking and cautious groping, and that where styrenes are concerned the clarification of a single detail often requires much time and effort.

The nature and extent of this problem become fully evident only when we remember that it is still not solved even when we have determined the structure of the addition products. The structural formulae of the molecules are merely simplified projections symbolizing the succession of the atoms and the way in which they are linked together. As far as their arrangement in space is concerned they tell us very little until we apply the principles of stereochemistry to them.

In the case of diene synthesis of styrene with maleic anhydride, which we have just been describing, the problem is then as follows: The addition product (LV) has seven asymmetrical carbon atoms which are of unequal valency

owing to the absence of certain molecular symmetrical forms and which are therefore capable of giving rise to the full number of $N = 2^7 = 128$ stereoisomers. These constitute 64 pairs of enantiomorphous forms – racemic compounds. Even when allowance is made for the fact that – in the light of a special stereochemical experiment*, details of which will not be discussed here – in our case the number of isomers is again reduced by half, 32 isomers are still conceivable.

For the second structural isomer (LVI) with four centres of asymmetry, similar considerations give the impressive number of 16 isomers. In all, therefore, no less than 32 + 8 racemic forms are possible within the framework of the established structural formula. Experimentally we found three – one belonging to structural type (LV), and two to (LVI).

These examples illustrate three-dimensionally a characteristic feature of diene synthesis, a feature which is linked in the most intimate way with its nature – namely its pronounced steric selective properties. Of the reaction courses which are formally possible, only a few are taken, and frequently only one.

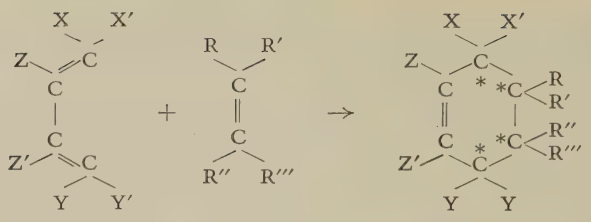
This peculiarity is one of the decisive factors determining the value of diene synthesis as a method. The fact that it can be used as a means of isolating and separating substances from mixtures, and that it is uniquely suitable as a means of determining the character of specific types of substance, could otherwise never have been known, for even the most highly developed methods of separating substances would be bound to fail in face of the difficulty of the problem to be solved here.

Diene Synthesis as a Stereochemical Phenomenon

The above examples of the peculiar steric nature of diene synthesis are better suited – since two successive additions are involved – to a definition rather than to a solution of the problem in question.

The steric preference for certain reaction courses among the many normally left open by the structural formula is a universal characteristic of diene synthesis, the peculiar nature of which we can see from the basic structural formula. This representation is simple and at the same time has the merit of maximum universality:

* Trans-«bridge»-links are out of the question as they could not exist.



(LVII) (LVIII) (LIX)

Upon addition of diene and philodiene the four addition centres usually become asymmetrical carbon atoms, which are of different valencies among themselves, and which formally can give rise to $2^4 = 16$ stereoisomeric addition products or eight pairs of enantiomorphous forms (racemic compounds)*.

These can be symbolized very clearly. The carbon ring formed by diene synthesis describes very roughly a plane, which we shall represent as a horizontal line. We then get the following eight possibilities (LX, 1–8) for the distribution of the substituents X, X′, Y, Y′, R, R′, R″ and R‴ on both sides of the ring plane**:

$$\overline{\underline{X}}\left\{\frac{R\ R''}{R'\ R'''}\right\}\overline{\underline{Y}} \quad \overline{\underline{X}}\left\{\frac{R'\ R''}{R\ R'''}\right\}\overline{\underline{Y}} \quad \overline{\underline{X}}\left\{\frac{R\ R'''}{R'\ R''}\right\}\overline{\underline{Y}} \quad \overline{\underline{X}}\left\{\frac{R'\ R'''}{R\ R''}\right\}\overline{\underline{Y}}$$

(1) (2) (3) (4)

$$\overline{\underline{X}'}\left\{\frac{R\ R''}{R'\ R'''}\right\}\overline{\underline{Y}'} \quad \overline{\underline{X}'}\left\{\frac{R'\ R''}{R\ R'''}\right\}\overline{\underline{Y}'} \quad \overline{\underline{X}'}\left\{\frac{R\ R'''}{R'\ R''}\right\}\overline{\underline{Y}'} \quad \overline{\underline{X}'}\left\{\frac{R'\ R'''}{R\ R''}\right\}\overline{\underline{Y}'}$$

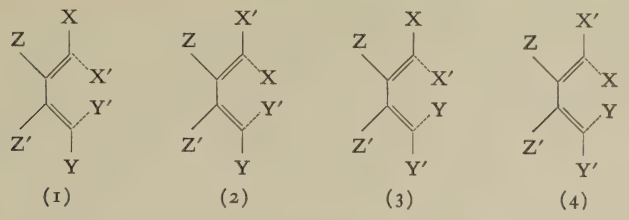
(5) (6) (7) (8)

(LX)

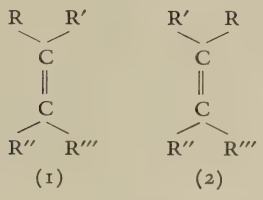
As is well known, the two components – diene and philodiene – (which have just formed) occur, in their turn, in various geometrical isomers: the diene in the most universal case in four (LXI, 1–4), the philodiene in two (LXII, 1–2).

* This number is reduced when substituents are identical with one another. It increases when the diene and/or the philodiene are carriers of further centres of asymmetry. In this case the steric problems of diene synthesis touch those of asymmetrical synthesis[24].

** Owing to the non-existence of a «*trans*-cyclohexane» the substituents Z and Z′ do not normally contribute to the formation of steric forms unless they possess their own centres of isomerism.



(LXI)



(LXII)

The problem of the steric course of diene synthesis boils down to the following three questions:

1. Does a relationship exist between the configuration of the philodiene and the arrangement of its substituents in the addition product?
2. Is there a similar relationship in the case of the diene component?
3. What relations apply to the mutual orientation of the two partners?

The «cis»-principle

A clear answer to the first question is given by comprehensive factual data.

The designation *cis* and *trans* for configurations in the olefine, characterizes the position of the substituents R, R′, R″ and R‴ relative to the double bond. By the addition of a diene, this double bond is eliminated and the two carbon atoms, which it links together, become parts of a ring. Experience shows that the relative position of the substituents remains unchanged during this process. It is the same in relation to the ring as previously in relation to the double bond. One of the forms (LX, 1, 4, 5, 8) regularly arises from the isomers (LXII, 1), whereas (LXII, 2) can only result in the types (LX, 2, 3, 6, 7), but not vice versa.

From this unequivocal correlation in the configurations of philodiene and addition product, it can be inferred that diene synthesis constitutes a pure *cis*-addition which takes place at the double bond of the philodiene (*cis*-principle)[23,24].

Retention of the diene configuration

Considerations similar to those just described in the case of the philodiene components can be transferred – mutatis mutandis – to the diene. As in the former case, *cis* and *trans* additions are formally possible with the 1,4-addition in the latter case, just as they are with the 1,2-addition in the former case, and should result in addition products of different configuration. Our experiments showed that here, too, the addition of the philodiene to the diene takes place in accordance with the *cis*-addition formula, and that therefore an analogous steric retention principle applies here:

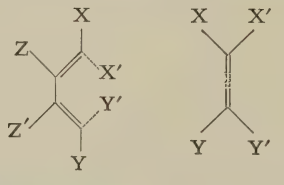
The diene enters the addition product with unaltered configuration, i.e. both *cis* substituents of the diene and both its *trans* substituents are situated on the same side of the ring in the addition product*. In the case of the diene (LXI, 1), for instance, only the forms (LX, 1–4) of the addition product satisfy this condition, the rest (LX, 5–8) conflict with it.

Whereas the *cis* principle, which was formulated at an early date, is now[24] supported by a vast body of experimental evidence, it is only recently[25] – owing to the inaccessibility of stereoisomeric dienes – that we have succeeded in confirming in a few characteristic cases, the « principle of the retention of the diene configuration ».

Diene synthesis and free rotation

In the preceding section we made a tacit assumption which requires some justification. As is well known, in classical stereochemistry, the principle of «free rotation» was postulated in order that the number of asymmetrical ethane derivatives demanded by the model might be kept in accord with

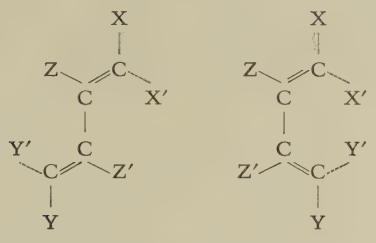
* The fact that the position of the substituents X′ and Y′ on the one hand, and that of X and Y on the other, are spatially equivalent, can probably be seen best if one imagines



that carbon atoms *2 and 3* of the diene are taken out, and the two remaining parts of the diene are reunited. Both pairs then assume a *cis*-position relative to one another. In this way the analogy of the *cis*-principle with the principle of retention of the diene configuration becomes particularly clear.

empirical findings. According to this principle carbon atoms linked singly with one another can rotate round the C–C axis.

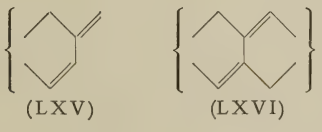
In the case of the diene component this would mean that between the two planar configurations – the « bent » one (LXIV) with addition centres as close together as possible, and the « stretched » one (LXIII) – all possible nonplanar transitions are conceivable:



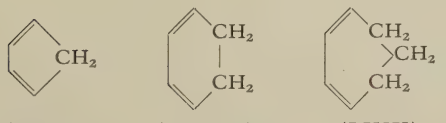
trans-arrangement (LXIII) *cis*-arrangement (LXIV)

So far, we have been assuming that diene synthesis takes place from arrangement (LXIV). There are several different arguments for this hypothesis, some of which we will now cite[26].

By means of constitutional factors it is possible to eliminate « free rotation » and also to define the « stretched » as well as the « bent » arrangement. It is found here that the first category, (LXV) and (LXVI), is unable to add philodienes, whereas compounds such as (LXVII–LXIX) are capable of diene synthesis:



(LXV) (LXVI)

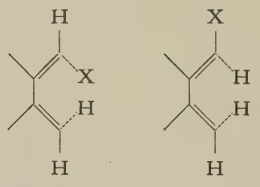


(LXVII) (LXVIII) (LXIX)

Significantly the reactivity optimum is with five-membered rings, which, thanks to their valency angle, by their very nature represent ideally the rigid planar diene. As the distance increases, so – according to the model – the mobility, and with it the possibility of deviation from the planar arrangement, increases. At the same time the reactive capacity decreases.

From a host of further arguments, I will point out that voluminous substituents Z and Z′, as well as a blocking X′ (or Y′) are capable of suppressing approximation to the «bent» planar form (LXIV), which is necessary for addition, to such an extent that addition no longer occurs.

In this connection the behaviour of the two series (LXX) and (LXXI) is informative:



(LXX) (LXXI)

X= —CH_3, —C_2H_5, —CH_2—CH_2—CH_3, —$CH(CH_3)_2$,

—$C(CH_3)_3$, —C_6H_5

With the *cis*-diene type (LXX) the capacity for addition declines as the branching and space-filling of the substituent X increase. According to empirical findings to-date it is totally eliminated with *tert.*-butyl and with phenyl. Significantly the analogous *trans*-dienes (LXXI) are without exception capable of diene synthesis, and the facility with which they react is substantially the same in all cases within this group. It is greater than with all analogues of the *cis*-series (LXX). The two hydrogen atoms in the *cis* position of (LXXI) obviously hinder least the coming into being of the «bent» arrangement, which is necessary for the reaction.

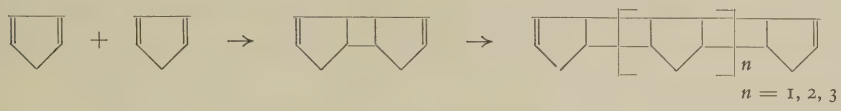
In order to express the formal similarity of the acyclic dienes in their bent arrangement to the cyclic dienes, e.g. (LXVIII), we have called the former «quasicyclic». The relations existing between free rotation and the course of diene synthesis can be reduced to the simple formula: cyclic and acyclic dienes show the same behaviour during diene synthesis, since the acyclic dienes come to reaction in their «quasicyclic» arrangement[26].

The universal orientation formula

In their simplicity, the steric rules so far described may to some extent be

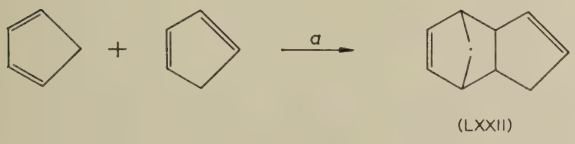
apparent if not perfectly obvious. However, this is not the case with the relationship now about to be discussed, which deals with the question of whether the two partners are orientated in relation to one another. A long and difficult journey had to be travelled before this steric law, the «universal orientation formula», was recognized and proved. Within the framework of this lecture I can only sketch very briefly a few of the stages on this road.

Its starting-point was the study of thermal polymerization of cyclopentadiene[27]. When heated, this hydrocarbon – as had been shown earlier – yields a polymerically homologous series, to whose formation the following uniform law seemed to apply:

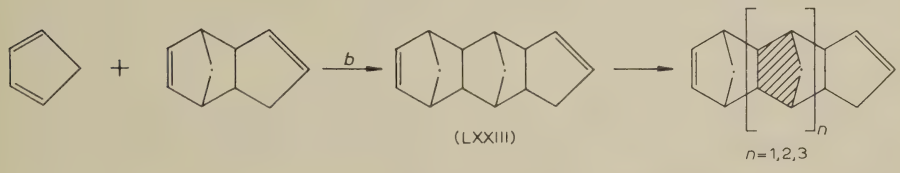


$n = 1, 2, 3$

Our first contribution consisted of a review of this fascinatingly simple structural formula. The principle of the formation of four-membered rings had to be replaced by the formula for diene synthesis. The polymerization of cyclopentadiene should be formulated as follows:



(LXXII)



(LXXIII)

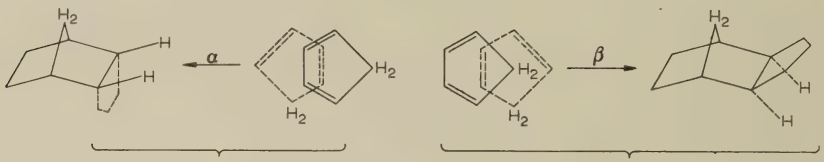
$n = 1, 2, 3$

Here for the first time it was found necessary to include the stereochemical as well as the structural aspect in the study of diene synthesis. This was occasioned by the isolation of two trimeric cyclopentadienes (LXXIII). Breakdown of these led to the important conclusion that the two trimers were already based on two dimers (LXXII), or in other words that process *a* must already occur in two directions.

These two directions (α and β) are foreseen by the model, and this question could have been regarded as settled with the isolation of a second β-dicyclopentadiene in addition to the α-form, which had long since been known,

and with the correlation of both with the configuration given by the model. That it was not, is due to one particular circumstance. The transitions which have been described take place at elevated temperature. The picture is very much simplified when one studies the changes undergone by monomeric cyclopentadiene at room temperature. The liquid hydrocarbon changes into the camphor-like mass of α-dicyclopentadiene within a few days. Structurally and sterically this process is completely uniform. Structurally it means nothing more than the first phase (*a*) of the above-described formation of a polymerically homologous series which at room temperature breaks off after the first member.

Its steric uniformity, however, inevitably seemed extremely remarkable, since it was obvious that only one of the possible courses α and β was taken at low temperature.



(LXXII, α) (LXXII, β)

Experiments soon convinced us that this phenomenon was by no means confined to dimerization of cyclopentadiene. Thus, addition of cyclopentadiene to maleic anhydride proceeds in only one direction sterically, and this direction is demonstrably the same as that found with dimerization of cyclopentadiene.

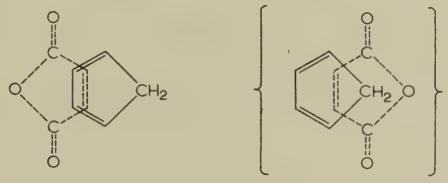
With regard to the question whether α or β was the preferred steric course, a very precise answer in favour of α could be given from the addition product – cyclopentadiene and maleic anhydride.

A comparison between the addition products formed in accordance with formula α and formula β shows quite clearly that the above-explained retention principles apply in both cases, and that consequently they alone are not sufficient to account for the observed steric selection.

On the contrary, a new selection principle must be operating here, a principle which can with advantage be described with the aid of « orientation complexes ». If one imagines the two partners – diene and philodiene – so close to one another that the four centres of addition* are situated on a

* Or, to be more accurate, their projection on the drawing-plane.

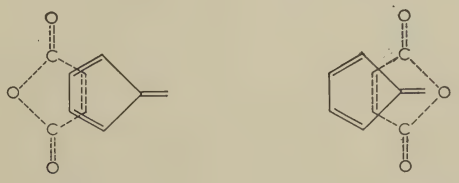
straight line, one recognizes that with α all the double bonds come to lie on one side of this line, whereas with β they are distributed on both sides. To put it another way: with diene synthesis, addition is entirely or predominantly steric when it is from an orientation complex with maximum accumulation of the double-bond systems of both partners (LXXII, α), but is entirely or predominantly non-steric when it is from the minimum-density arrangement of these systems (LXXII, β)[23].



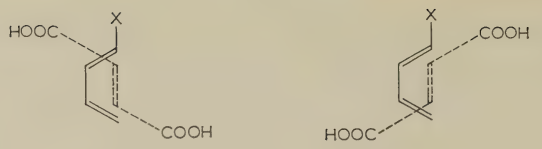
(LXXIV, α) (LXXIV, β)

This statement holds good in a wide range of cases. We deduced it for many diene syntheses, in the first place in very great detail for the additions of maleic anhydride, which – for instance, with cyclopentadiene – always adds in accordance with formula (LXXIV, α) and not in accordance with (LXXIV, β). This picture is not materially changed if we go from the cyclic to the acyclic dienes, since these, as explained in the preceding section, react in their « quasicyclic » arrangement. The same applies if we go from maleic anhydride to acrylic acid, or to philodienes of the vinyl acetate type, where the uncombined electron pairs obviously assume the function of the double bond.

On the other hand, as was to be expected, the « universal orientation formula » ceases to be valid where the demand for maximum accumulation of the double-bond systems of both partners ceases to be unequivocal, which is the case in the diene class, e.g. with the fulvenes (LXXV, α and β), and in the philodiene series, e.g. with the diene syntheses of fumaric acid (LXXVI, α and β).



(LXXV, α) (LXXV, β)

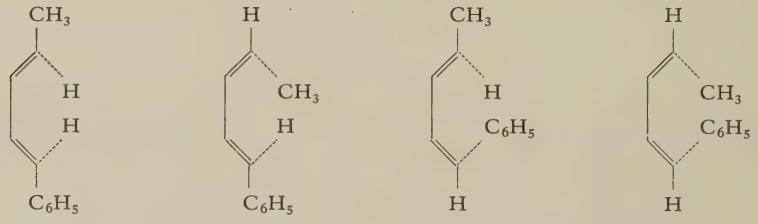


(LXXVI,α) (LXXVI,β)

Here neither of the two orientation complexes is distinguished from the other, and the addition is steric in both directions.

An example

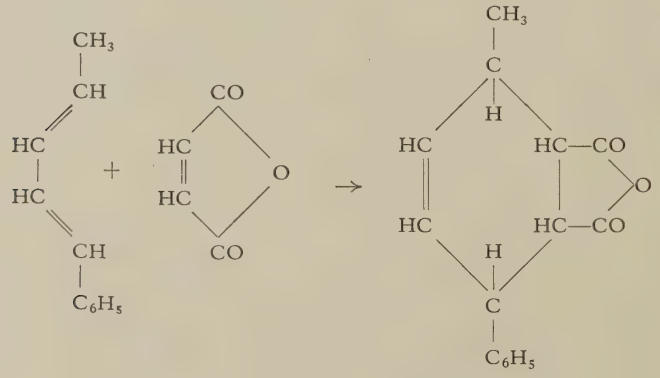
1-Phenyl-4-methylbutadiene exists in four forms (*a–d*), of which three (*a–c*) are well known.



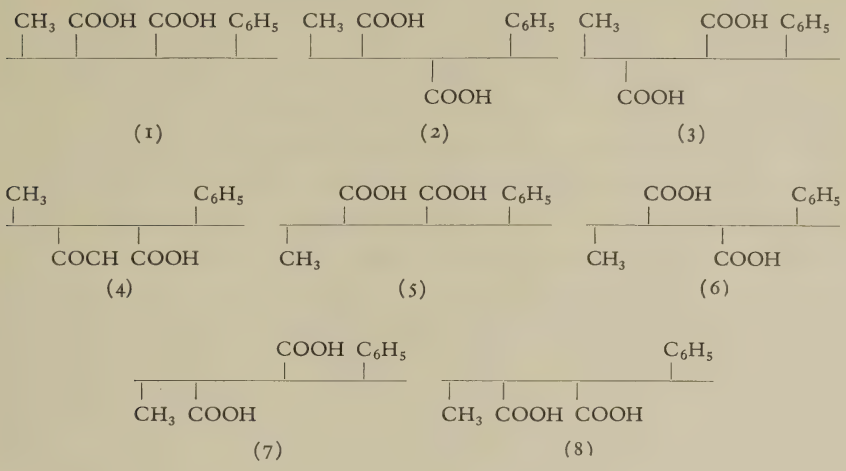
(*a*) (*b*) (*c*) (*d*)

As is to be expected from our empirical findings, form (*c*) can enter into no diene synthesis, since the phenyl group prevents the necessary approximation to the planar arrangement. Both forms (*a*) and (*b*) are capable of diene syntheses, and, as we know from experience, (*a*) participates more readily in these reactions than does (*b*).

Structurally diene synthesis of 1-phenyl-4-methylbutadiene with maleic anhydride or fumaric acid presents the following picture:



Sterically the forms (1–8) are possible.



Case I: *trans*, *trans*-form (*a*), and maleic anhydride.

The *cis*-principle, which demands retention of the philodiene configuration, rules out (2), (3), (6), and (7); (5), (6), (7), and (8) are incompatible with the principle of retention of the diene configuration in the addition product. Of the remaining alternatives (1) and (4), the universal orientation formula decides in favour of (1). In accord with empirical findings, only one of the eight possible forms – namely the *all-cis* form (1) – arises.

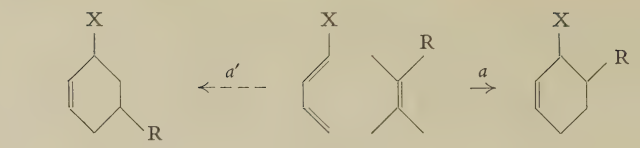
Case II: *trans*, *cis*-form (*b*), and maleic anhydride.

In this case, by the same reasoning it can be predicted that form (5) will be the reaction product, in accord with empirical findings.

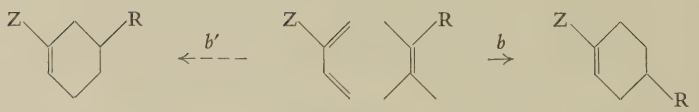
Case III: *trans*, *trans*-form (*a*), and fumaric acid ester yield, as predictable, forms (2) and (3) side by side.

Diene syntheses with asymmetrical addenda

The above steric interpretation does not conclude the discussion of the universal structural formula for diene synthesis. On the contrary, the structural formula confronts us with a further question: What course do diene syntheses take when the structural asymmetry of the two partners permits two alternative reactions? The two following combinations of an asymmetrical philodiene with a diene substituted in the α- or β-position represent the simplest processes of this type:



(LXXVII)



(LXXVIII)

The empirical data recently obtained from these and more complex examples show selectivity again here, in that in the first case ortho-forms (*a*) and in the second para-forms (*b*) tend to arise before formation of meta-types (*a'*, *b'*), since these types occur only as by-products, if at all.

In the light of our experience to-date, these findings, and this is most remarkable, are largely independent of the nature of the substituents X and R.

From the many results to hand I should like to point out one in particular which is very informative, namely the addition of *trans*-butadiene-1-carboxylic acid (X = COOH) to acrylic acid (R = COOH). Despite the polar character of the same substituents, structurally they move in close to one another and yield the ortho-form (LXXVII). This clustering of two groups of equal polarity is as complete here as possible, for it is found that – entirely in accordance with the steric rules described above – the *cis*-form of the ortho-series is the main product of this diene synthesis[24].

Observations on the mechanism of diene synthesis

Diene synthesis of butadiene-1-carboxylic acid with acrylic acid is in no way an exceptional case as far as its course is concerned. On the contrary, we have encountered other cases where groups of equal polarity crowd in as close as possible together during addition. I draw particular attention to this fact here because it provides me with an occasion for a remark on the mechanism of diene synthesis.

So far I have confined myself to the means of expression of classical structural chemistry – valency line, double bond, and asymmetrical carbon atom – in my descriptions of results. The fascinating simplicity of the new reaction, long ago prompted experiments with a view to a definition of it in the light of the modern theory of linkage[28].

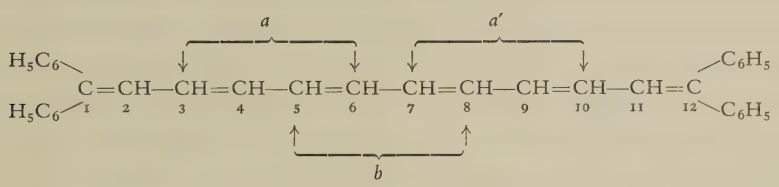
At first it was suggested that diene synthesis was a (crypto-) ionic process. It was thought that displacements of the charge both in the system of the diene and also in that of the philodiene, induced polarities, and that the driving force for their union lay in their intermolecular equilibrium.

Our experimental results, obtained during a study of asymmetrical addenda, showed this interpretation of diene synthesis to be highly improbable – at least that it is not the determining factor governing the course of the addition process. The same nature of substituents X and R and their same position in the double-bond system of both partners ought to induce the same charge formula in both, and the equilibrium of this formula ought inevitably to give the same meta-type. This is obviously at variance with experimental findings.

The fact that polar solvents and catalysts have relatively little effect on diene syntheses likewise points away from an ion reaction mechanism.

The strongest argument against such a mechanism lies in the fact that it in no way appears to obey the laws which govern diene synthesis structurally and sterically.

Structurally it is the formation of six-membered rings, the laws governing these being particularly striking where there is an opportunity for rings of relatively large width to arise. Since first being posed, with regard to the polyenes[9], this problem has been repeatedly examined – on the last occasion[10] by means of addition of maleic anhydride to 1,1,12,12-tetraphenyldodecahexaene.



As is indicated by the sketch of the addition centres in the 3,6- and the 7,10-position on the one hand (*a*, *a'*) and in the 5,8-position on the other (*b*), the 6-ring formation also dominates the field here. The (crypto-) ionic interpretation does not account for this phenomenon; on the contrary, the regular formation of six-membered rings directs our attention to another relationship.

From the point of view of the electron theory of valency, the total balance-sheet of diene synthesis lies in an interplay between the functions of the

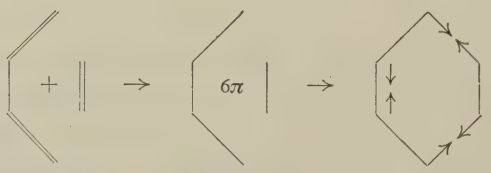
six π-electrons of the two partners. Two of them retain – although in a changed position – their character as π-electrons, whereas the remaining four are used in pairs for the formation of two σ-bonds in the 6-ring. Both factors, i.e. the six π-electrons and the six-membered carbon ring, point to similarities between the linkage system of benzene and that of the aromatic hydrocarbons.

As is well known, compared with a cyclohexatriene with double bonds visualized as being localized, benzene is a relatively stable structure from the chemical and the energy aspect. Extensive experimental work has shown this « aromatic character » to be confined to the number 6, and according to the quantum theory it can be ascribed to a closed inner sextet of bonding π-electrons.

An identical – or at least very similar – group of six bonding electrons of « aromatic resonance » is by all appearances also at work during diene synthesis; indeed it is thought to be the triggering factor. As the two partners – diene and philodiene – approach one another, the electron sextet forms as a result of reciprocal decoupling of their π-electron systems, the energy required for this decoupling being supplied from the gain in resonance energy.

We can therefore supplement the universal reaction formula for diene synthesis by inserting an unstable complex-type structure (LXXX) as a transition between the initial system of the two partners (LXXIX) and the final stage of the stable product of addition (LXXXI).

Since the components already occupy spatially well-defined positions in this structure, we named these intermediate combinations « orientating complexes ». The cohesion is effected by a sextet of electrons supplied jointly and proportionally to both partners. The conversion of this sextet into two σ-bonds, which complete cyclization, and into a double bond, which occupies a specific position, brings the addition process to a close.



(LXXIX) (LXXX) (LXXXI)

On the basis of this hypothesis diene synthesis is not a (crypto-) ionic but a (crypto-) radical process where the course of the reaction is determined by those factors which are decisive in the decoupling of electron pairs. Inductive

effects emanating from polar groups and manifesting themselves as charge displacements through the carbon chains will undoubtedly be present, although, being forces of relatively low magnitude, they will not usually be decisive – in complete contrast to the resonance forces exerted by multiple bonds upon one another or in their interplay with non-proportionate electron pairs of polar groups. I must confine myself here to stating that among the laws characterizing the picture of diene synthesis, none has yet been found which conflicts with the interpretation put forward above.

I am aware that this rough sketch merely suggests the direction in which an explanation of the peculiarities of diene synthesis is probably to be sought. Its structural and steric selectivity makes possible an enormous number of π-electron systems of both partners – and in fact not only of the substituents participating directly in the addition process but also those of «activating» substituents. This fact will have to be the starting-point of a detailed discussion of the configuration of the π-electron systems in the orientating complexes.

Diene Synthesis and Substituting Addition as a Special Case among General Reaction Types

Over the 23-year period during which diene synthesis was being developed, a vast body of experimental data accumulated, but despite the variety and abundance of individual cases, certain general laws are discernible among all the results obtained. These laws are clearly the expression of the fact that these are pure and direct interactions between electron systems of two partners – in other words, effects which are not complicated by the participation of accessory agents.

It is not surprising that processes of this type should not be confined to pure carbon systems but that other arrangements of atoms with suitable electron configurations show a tendency towards similar conversions. Cases of this type have become known in increasing numbers, so far primarily with the elements oxygen and nitrogen. Thus, instead of the arrangement C=C—C=C, the groups C=C—C=O and N=C—C=N can also act as dienes, whereas in addition to the classical philodienes, >C=C< and —C≡C—, combinations such as >C=O, —N=N—, —C=N, and —N=O are capable of fundamentally the same effects. This second group is rounded off by molecular oxygen. Developments during the past few years have shown

ever more clearly the similarity of this substance to maleic anhydride in its reactions with dienes and olefines. The merging of diene synthesis and of substituting addition, on the one hand, with the apparently completely heterogeneous phenomena of autoxidation, on the other, into a homogeneous group of processes will presumably be only one stage in this development. It is very probable that in the majority of cases the additions of diazomethane, of diazoacetic acid, of organic azides, etc., to unsaturated systems, are triggered off and are determined, as far as their course is concerned, by resonance phenomena between the π-electron systems of the two partners The task now is to investigate such effects of unsaturated substances upon one another from aspects which have become evident for the first time during the study of diene synthesis as the ideal type of this reaction.

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Biography

Kurt Alder was born in Königshütte, Upper Silesia, on the 10th of July 1902. His childhood and school years were spent in these industrial surroundings, but after the end of the First World War he was forced to leave his home, due to political circumstances.

He started reading chemistry at Berlin University in 1922, and later continued these studies at Kiel, where he obtained his degree of Ph.D. in 1926. The thesis for the doctorate, on which Alder worked under O. Diels, was entitled: *Über die Ursachen der Azoester-reaktion* (On the causes of the azoester reaction).

In 1930 Alder was appointed reader for chemistry by the Faculty of Philosophy at Kiel University; promotion to lecturer followed in 1934. Alder left Kiel in 1936 to take up the appointment as head of department in the science laboratories of the I. G. Farben-Industrie, at their works in Leverkusen, where he worked on the preparation and constitution of synthetic rubber («Buna»). By this work some of his earlier interests were reawakened and stimulated.

In 1940 Alder was appointed to the Chair for Experimental Chemistry and Chemical Technology at Cologne University and also became Principal of the Institute of Chemistry. He received invitations from Berlin University in 1944 and from the University of Marburg in 1950, but declined both.

As early as 1927–1928, whilst at Kiel, Alder had studied problems of systematic organic chemistry in collaboration with his teacher O. Diels, and this lead to their joint discovery of the principle of the diene-synthesis, which they investigated and determined in all its aspects. At the same time Alder also worked in collaboration with younger colleagues on extensive stereochemical investigations, prompted by selection phenomena during organic chemical reactions, particularly in unsaturated systems. A series of other problems, such as the behaviour of double bonds in stressed carbon rings and the phenomena of intermolecular rearrangements, were investigated.

Although conditions in Cologne during the 1940's were not favourable

for scientific research, Alder was nevertheless able to continue his original work systematically and even discover relationships which were decisive for future developments. These are characterized by the transition from pure additive processes, of which the diene-synthesis is the most important, to processes of substitution. The purpose of these investigations was the analysis and the elimination of the dualism existing between addition and substitution. These studies covered a wide field and include also the reaction of molecular oxygen on unsaturated substrates.

Kurt Alder's investigations have been described in about 150 papers, which were published mainly in *Justus Liebig's Annalen der Chemie*, in the *Berichte der Deutschen Chemischen Gesellschaft* and in *Angewandte Chemie*.

In recognition of his work, Alder received the Emil Fischer Memorial Medal from the Association of German Chemists, in 1938. In the same year he became a member of the Kaiserlich Leopold.-Karol.-Deutsche Akademie der Naturforscher (Imperial Leopold.-Karol.-German Academy of Natural Philosophers) in Halle. The Medical Faculty of the University of Cologne conferred the honorary degree of M.D. on Alder in 1950, and in 1954 he received the honorary doctorate of the University of Salamanca.
Kurt Alder died on the 20th June 1958.

Chemistry 1951

EDWIN MATTISON MCMILLAN

GLENN THEODORE SEABORG

«for their discoveries in the chemistry of the transuranium elements»

Chemistry 1951

Presentation Speech by Professor A. Westgren, Chairman of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Your Royal Highnesses, Ladies and Gentlemen.

In his famous treatise on air and fire, published in 1777, Scheele writes that in some quarters at that time it was regarded as futile to make any more research into what elements bodies might consist of. « A depressing prospect » he adds, « for those whose greatest pleasure it is to study the composition of substances found in nature. » Scheele's own experience and the subsequent developments up to our day have shown that, at the end of the 18th century, there certainly still was enough to do for those who wanted to discover new elements. At least as many elements as were then known still remained to be discovered.

In 1794, Scheele's friend from his days in Uppsala, the Åbo professor Johan Gadolin published in the proceedings of the Academy of Sciences a report on a « Study of a black heavy kind of stone from Ytterby Stone Quarry at Roslagen ». In this mineral – later called gadolinite, after him – he had found a hitherto unknown earth, the so-called yttria. Nine years later Berzelius, in a mineral from Riddarhyttan in Västmanland (the so-called « Bastnäs tungsten ») discovered another earth, ceria.

These two discoveries together provided the starting-point for studies of the so-called rare-earth elements which went on throughout the 19th century. Already Gadolin had reckoned with the possibility that the yttria isolated by him was not a simple substance and it proved indeed later to consist of several oxides. Berzelius' ceria turned also out to be a mixture. The separation of the different components in these compound earths has been no easy task, since they are chemically very similar to one another. Little by little, however, it has been possible to divide them up completely, and within this group alone as many as 14 different elements have been isolated. Swedish chemists, chief among them being Mosander and Cleve, have made very valuable contributions in this domain of chemistry. Of the rare-earth metals many – yttrium, terbium, erbium, ytterbium, scandium, thulium, holmium – have been given names that show their origin in various Swedish localities.

Besides this group of closely connected rare-earth metals many other ele-

ments were discovered in the course of the 19th century. A comprehensive survey of all the known elements was provided in 1869 by the establishment of the Periodic System. At that time Mendeleev and Lothar Meyer independently discovered that there were clear evidences of periodicity in the chemical character of the elements when they were arranged in the order of increasing atomic weights. From this regularity Mendeleev was able to conclude that certain gaps remained still to be filled, and he could even predict all the most important properties of these still undiscovered elements and their compounds. His predictions have been fully confirmed by later discoveries.

During the years around 1920, Niels Bohr's investigations on the structure of atoms threw new light on the Periodic System. It was now possible, among other things, to explain the chemical similarity between the rare-earth elements. The positive charge in the nucleus of the atom and the number of electrons surrounding it rises by one unit for every step upwards in the element series. This additional electron usually forms part of the outermost shell of the atom, and since the chemical characteristics depend on the structure of the atom in just this part, the successive members in the series of elements can for the most part be clearly distinguished from one another in respect to their chemical properties. But within the group of the rare earths it is not the outermost electronic shell that is developed, nor the shell beneath it, but the one that underlies that.

The result is that, through the whole series of these elements, the exterior parts of the atomic structure remain virtually unchanged. Together they come to form what might be called a group of quasi-isotopes. Since they are like lanthanum, the first element in the series, they have been given the comprehensive name of lanthanides.

If, said Bohr, there existed an extension of the series of elements beyond the heaviest of them all, Nr. 92, uranium, then this would form a new series of very closely associated elements. They would all resemble uranium and, by analogy with the lanthanides, would form a series of uranides.

By experiments which were carried out during the years 1936–1938, Otto Hahn and Lise Meitner believed they could confirm Fermi's statement that the transuranium elements are formed by irradiating the heaviest elements with neutrons. But these synthetic elements were not like uranium, but appeared to be homologues of elements so dissimilar to one another as rhenium, the platinum metals, and gold. Hahn and Strassmann made, however, late in 1938, the epoch-making discovery that is was not really a question of

transuranium elements at all here. The heavy atoms were found to split up into substances belonging to the middle of the elemental series and this brought the whole problem into a new stage.

The first transuranium element of which there was definite proof was produced by McMillan and Abelson in May 1940 at the University of California, by irradiating uranium with neutrons with the aid of the cyclotron built by Lawrence. It was obtained as a disintegration product of a β-radiating uranium isotope, which has a half-life of 23 minutes. Hahn and Meitner had also discovered this body, but their preparation was too weak for its daughter-product to be demonstrated. The Americans were able to investigate this thoroughly, and showed that it forms an isotope of element 93, that is to say, a transuranium element. They called it *neptunium* after the planet Neptune, whose orbit lies next outwards after Uranus in the solar system. By irradiating uranium with rapid neutrons or with heavy-hydrogen nuclei, deuterons, other neptunium isotopes were soon produced in Berkeley.

In 1940 McMillan and Seaborg and their fellow-workers had already reported that when neptunium disintegrates it gives rise to an element 94. By analogy with the way in which names had been found for neptunium and uranium, this second transuranium element was called *plutonium*, after the planet Pluto, which has its orbit next outside that of Neptune. The first isotope of this element, which has a half-period of 24,000 years and thus is relatively stable, is what is called an atomic fuel. This plutonium isotope reacts with slow neutrons in the same way as the uranium isotope ^{235}U, that is to say, when it is split it develops great energy and gives off neutrons. In this way it came to play an important part in the atomic-bomb project during the war, and methods were developed for its production on a large scale.

After these problems, conditioned by the war, had been solved, Seaborg, as leader of a comprehensive group of able colleagues, completed the studies of the transuranium elements. In doing this, he has written one of the most brilliant pages in the history of the discovery of chemical elements.

Not less than four more transuranium elements have been produced. The chemical characteristics of all these new elements have been established by developing a refined ultra-microchemical experimental technique. Bohr's prophesy that in the transuranium elements we are dealing with a group of substances of the same sort as the rare-earth metals, has thus been confirmed. However, this new series of closely associated elements does not begin with uranium 92, but with actinium 89. Thus, corresponding to the lanthanides,

there are the actinides, and a certain agreement can be found member for member between these two series. Seaborg therefore proposed for the new transuranium elements 95 and 96 the names *americium* and *curium*, in analogy with their corresponding rare earths europium and gadolinium (after Europe and Gadolin respectively). The two transuranium elements most recently discovered, berkelium and californium, correspond to terbium and dysprosium in the lanthanides.

By irradiating different sorts of heavy atoms with neutrons, protons, deuterons, helium nuclei, or, most recently, carbon nuclei, a great number of isotopes have been produced from the six transuranium elements. The study of these isotopes' formation and properties has yielded a wealth of scientific material.

A great many, originally isolated, observations on the radioactive transmutation series were made during the work on the great plutonium project. Thanks above all to Seaborg's activities it has been possible to bring these observations together into a comprehensive wholeness. In this way there was discovered an entirely new radioactive series which, from its most long-lived member, is now called the neptunium family.

The mass numbers of the three radioactive families which were previously known have the form $4n$ (thorium series), $4n + 2$ (uranium series) and $4n + 3$ (actinium series). Here the neptunium series fills a gap with mass numbers of the form $4n + 1$.

During his studies on the reaction of slow neutrons with thorium, Seaborg and his colleagues made a discovery which opened important technical prospects. They obtained a uranium isotope ^{233}U, which gives off α-rays and has a half-period of 120,000 years. This isotope, like ^{235}U, can be used as an atomic fuel. Thorium, which is more plentiful in nature than uranium, will therefore probably play a rôle as a basic material in the production of atomic energy.

The Swedish Academy of Sciences is of the opinion that these discoveries in the realm of the chemistry of the transuranium elements, of which I have here tried to give a brief account, are of such importance that McMillan and Seaborg have together earned the 1951 Nobel Prize for Chemistry.

Dr. McMillan. In 1934 Fermi showed that nuclear transmutations could be brought about by irradiating the heaviest elements with neutrons. Research into the reactions thus produced has, however, met with certain difficulties, and it took longer than was expected for the existence of the

transuranium elements to be proved. You were the first to succeed in this enterprise. By your discoveries you have opened a field of research in which vast and fundamentally important scientific and technical gains have been made. Later, by your work on the accelerator problem you have also actively furthered the progress in this domain of chemistry.

Dr. Seaborg. At a time when the possibilities of finding new elements appeared to be exhausted, you have produced a whole row of them and thus extended the Periodic System beyond the limits which, one might say, Nature seemed to have established. With great skill you have studied the chemical characteristics of the new-found elements, and so made clear their atomic structure.

In times past, the hunting for new elements has been a favourite occupation of many Swedish chemists and, to put it modestly, their efforts have not been in vain. Quite a number of elements still unknown in the days of Scheele have been discovered in this country. Such achievements have been appreciated here and it is but natural that we rejoice in the fact that again a man of Swedish blood has taken part, and this time a leading part, in fundamental and very successful work of this kind.

Gentlemen. While proffering you our Academy's warmest congratulations, I will now ask you to receive the Nobel Prize for Chemistry for 1951 from the hands of His Majesty the King.

Edwin M. McMillan

The transuranium elements: early history

Nobel Lecture, December 12, 1951

In this talk I shall tell of the circumstances that led to the discovery of neptunium, the first element beyond uranium, and the partial identification of plutonium, the next one beyond that. The part of the story that lies before 1939 has already been recountered here in the Nobel Lectures of Fermi and Hahn; I played no part in that and shall not repeat it now. Rather I shall start with the discovery of fission by Hahn and Strassmann. News of this momentous discovery reached Berkeley early in 1939. The staff of the Radiation Laboratory was put into a state of great excitement and several experiments of a nature designed to check and extend the announced results were started, using ionization chambers and pulse amplifiers, cloud chambers, chemical methods, and so forth.

I decided to do an experiment of a very simple kind. When a nucleus of uranium absorbs a neutron and fission takes place, the two resulting fragments fly apart with great violence, sufficient to propel them through air, or other matter, for some distance. This distance, called the « range », is a quantity of some interest, and I undertook to measure it by observing the depth of penetration of the fission fragments in a stack of thin aluminium foils. The fission fragments came from a thin layer of uranium oxide spread on a sheet of paper, and exposed to neutrons from a beryllium target bombarded by 8 MeV deuterons in the 37-inch cyclotron. The aluminum foils, each with a thickness of about half a milligram per square centimeter, were stacked like the pages of a book in immediate contact with the layer of uranium oxide. After exposure to the neutrons, the sheets of aluminum were separated and examined for radioactivity by means of an ionization chamber. The fission fragments of course are radioactive atoms, and their activity is found where they stop.

The result of the experiment is shown in the first slide (Fig. 1). The horizontal scale indicates the depth in the stack of foils, in terms of equivalent centimeters of air. The vertical scale indicates the activities of the foils, measured about two hours after the end of the neutron bombardment. The greatest depth of penetration and therefore the maximum range of the fragments

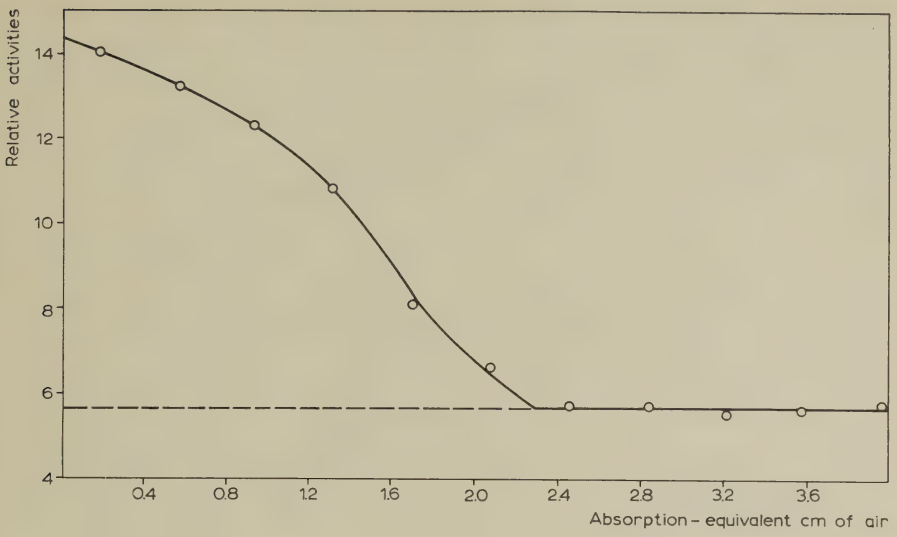

Fig. 1. Result of experiment to determine the range of fission fragments.

is seen to a little over two centimeters; the activity beyond this depth is that produced by the action of the neutrons on the aluminum itself, and it is seen to be nearly as great as the fission product activity on the first foil. There were however some interesting details of the fission process whose observation would be rendered difficult by this large background of activity. The fission fragments have various masses and therefore various ranges; is there any difference in the rate of decay of the long-range and short-range fragments? To find this out, I did a second experiment in which the foils were made of paper, a material which would not itself become radioactive. Since ordinary paper contains mineral matter, the backing for the uranium oxide was filter paper, and the thin foils were cigarette paper that had been extracted with acid. These were neither as thin nor as uniform as the aluminum foils, but that did not matter in this experiment.

Nothing very interesting about the fission fragments came out of this; the decay curves of the activities deposited on the various cigarette papers were about alike. However the filter paper, which held the uranium and at least half the fission products, showed something very interesting. Its decay curve was different; there was present a strong activity with a half-life of about 25 minutes, and another with a half-life of about two days. These lives could not be measured accurately because of the presence of part of the fission product activity in the same sample, but there was no doubt of their occur-

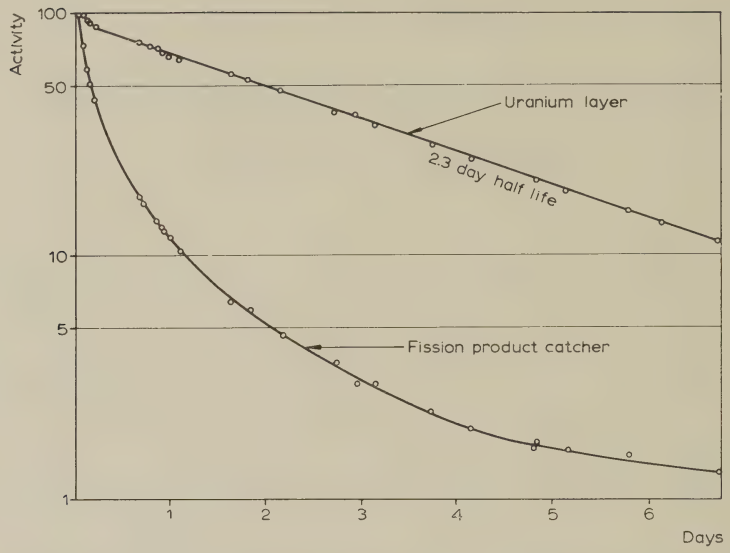

Fig. 2. Later part of decay curves of the activities found after neutron irradiation in a thin layer of $(NH_4)_2U_2O_7$, and in a sheet of cellophane («fission product catcher») which was next to that layer during the irradiation.

rence. The shorter period could with reasonable certainty be ascribed to the 23-minute uranium isotope U-239, discovered by Hahn, Meitner, and Strassmann in 1936. Since this is formed by simple neutron capture, it would not recoil out of the uranium-oxide layer like the fission fragments. The two-day period could then be the product of the beta-decay of U-239, and therefore an isotope of element 93; in fact this was its most reasonable explanation. However some time was to elapse before the proof of this would be given.

The original purpose of the experiments just described was not very profound and the measurement of the range was not very precise, but the by-product, the two-day period, was very interesting. Therefore the technique was improved so as to increase the yield and purity of the non-recoiling activities. Sheets of bakelite were used as a backing, and a thin uniform layer of ammonium uranate was deposited on these by allowing it to settle out of a suspension. After activation, the layer of uranium compound was scraped off and its activity was studied. The next two slides show the decay curve of the first sample made in this way, a week after the note describing the earlier results had been submitted to *The Physical Review*. The first of these (Fig. 2) shows the later part of the decay curve, indicating the presence of a single substance with a 2.3 day half-life. On the same plot is the decay curve of a

portion of the fission products caught on a sheet of cellophane placed next to the uranium; it shows the continuous curvature characteristic of the mixed fission products. The second (Fig. 3) shows the early part of the decay curve. The black dots are obtained by subtracting the 2.3-day activity, and give a value of 23 minutes for the short period, in exact agreement with the known period of U-239. The activity of the fission products caught on the cellophane is again shown.

When it thus became possible to prepare a new active substance in reasonably good purity by simple physical means, the question of its chemistry could be investigated, and Segrè undertook to do this. The expected chemical properties of element 93, according to the Periodic Table, were those of rhenium. Segrè was very familiar with the chemistry of this element, since he and his co-workers had discovered another of its homologs, now called technetium, in 1937; this was the first of the synthetic elements to be found. He showed that the 2.3-day material had none of the properties of rhenium, and indeed acted like a rare earth instead. Since rare earths are prominent

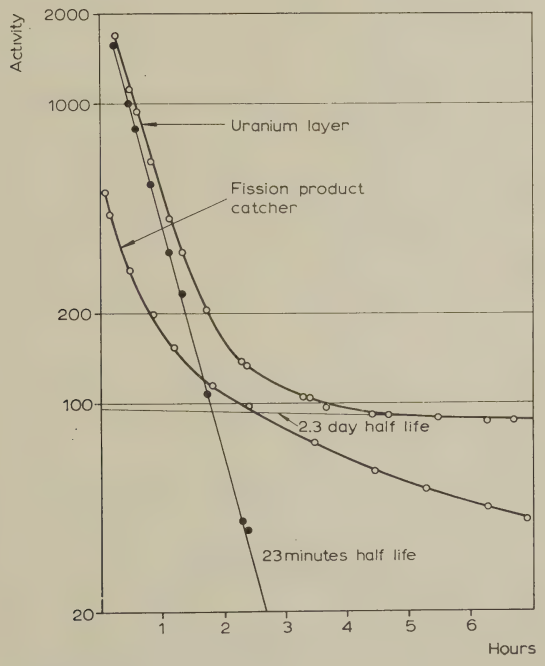

Fig. 3. Early part of same curves. The resolution of the «uranium layer» curve into 23-minute and 2.3-day periods is shown.

among the fission products, this discovery seemed at the time to end the story. However as time went on and the fission process became better understood, I found it increasingly difficult to believe that one fission product should behave in a way so different from the rest, and early in 1940 I returned to the problem. By this time the 60-inch cyclotron was running and deuterons of 16 MeV were available. Two physical experiments were illuminating. In one, the effect of cadmium, which absorbs thermal neutrons, was investigated. It reduced the fission product activity compared to the two non-recoiling activities, without changing the ratio of the latter. In the second, the earlier recoil experiments were repeated with a fission product catcher made of very thin collodion, and it was found that the range of the 2.3-day substance was certainly not greater than 0.1 millimeter of air. These great differences in behavior from the fission products made the interpretation of the 2.3-day activity as a rare earth almost impossible.

At this point I started to do some chemistry, and in spite of what the Nobel Committee may think, I am not a chemist. However I did find that the 2.3 period did not always follow the rare-earth chemistry consistently. For instance, in a fluoride precipitation with rare-earth carrier, sometimes only a part of the activity would come down, part remaining in solution. By now it was the spring of 1940, and Dr. Philip Abelson came to Berkeley for a short vacation. He had been a graduate student in the Radiation Laboratory, working on the uranium activities, when fission was announced, and in fact the method he was using would inevitably have led to his discovery of that phenomenon. After getting his degree he had gone to the Carnegie Institution of Washington, and was there, unknown to me, trying to separate the 2.3-day period chemically from large samples of uranium on the basis of

Six-valent uranium

ions: UO_2^{++}, UO_4^{--}, U_2O_7
compounds: UO_2F_2 is soluble.
$Na\,(UO_2)\,(C_2H_3O_2)_3$ is only slightly soluble.

Four-valent uranium

ion: U^{++++}
compounds: UF_4 is insoluble (like ThF_4, LaF_3, etc.).
$U\,(IO_3)_4$ is insoluble (like $Th\,(IO_3)_4$).

Fig. 4. Some pertinent chemical properties of uranium.

rare-earth-like properties. When he arrived for his vacation and our mutual interest became known to one another, we decided to work together. I prepared the 2.3-day substance by the method described earlier, and he tried some new chemical ideas, finding the correct approach very quickly. The key to the situation was the state of oxidation of the material. When in a reduced state, it precipitates with rare-earth fluorides; when in an oxidized state, it does not. In earlier work where the degree of oxidation was not controlled, erratic results are not surprising.

This suggests a strong similarity to uranium. The next slide (Fig. 4) illustrates some of the chemistry of uranium in water solution. The important valences are six and four; in the upper state it forms both positive and negative ions, uranyl and uranate, the transition between these being governed by the acidity of the solution; in the lower state it is like thorium or 4-valent cerium. In hydrofluoric acid, uranyl fluoride is soluble while uranium tetrafluoride is insoluble, like the fluorides of the rare earths. Thus the results with element 93 would be explained if it had the same valence states with some of the same properties as uranium, differing in the stability of the two states, the lower state being relatively more stable in element 93. The similarity with uranium was further demonstrated by the following results. Four-valent uranium, like thorium and four-valent cerium, is precipitated by iodate, while the trivalent rare earths are not; element 93 in the reduced state came down with thorium iodate. Six-valent uranium forms a remarkable compound, sodium uranyl acetate, which can be precipitated from a strong sodium acetate solution; element 93 in the oxidized state came down with this precipitate, a reaction previously thought to be characteristic of uranium alone among all the elements.

When the chemistry of element 93 was known well enough to separate it from all other elements, Abelson and I were in a position to demonstrate its growth from the 20-minute U-239. This was done by taking a series of successive rare-earth fluoride precipitates out of a chemically purified sample of the 23-minute uranium, in a reducing solution. The next slide (Fig. 5) shows the result. The initial activities of the samples, shown by the small circles, follow the 23-minute half-life of the parent substance, while each sample decays with a 2.3-day life, as indicated by the nearly horizontal lines at the right. With this proof of the genetic relation we could be sure that the 2.3-day substance was actually an isotope of element 93, which had escaped earlier identification only because its chemical properties are like those of its neighbor rather than that of its homolog. A similar phenomenon of chemical

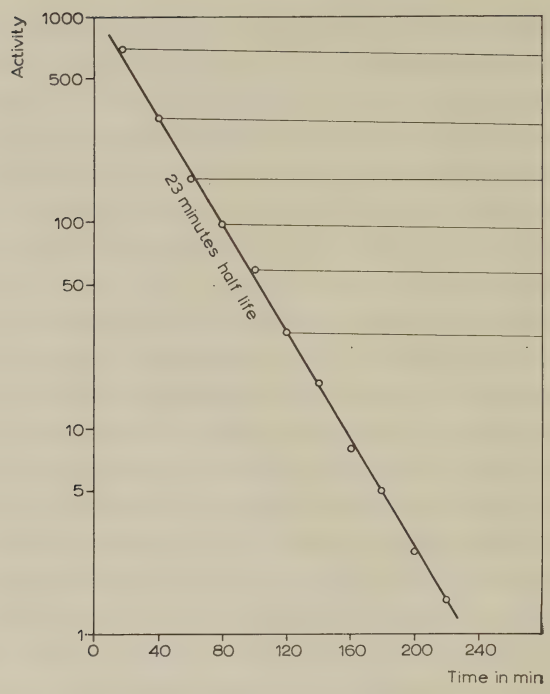

Fig. 5. Result of experiment to show the genetic relation between the 23-minute and 2.3-day substances.

similarity of neighbors occurs in the rare earths, and it was clear that this was happening again at element 93. The fact that some of the chemical properties of 4-valent 93 and 3-valent rare earths are alike is really only a coincidence; farther beyond uranium the elements actually become like the rare earths with a predominant 3-valent state, but of course Abelson and I did not know this at the time; when we spoke of a «second *rare-earth* group of similar elements» in our note in *The Physical Review*, we are thinking only of the phenomenon of similarity of neighbors.

Before submitting that note we tried to identify the product of the decay of the 2.3-day substance, which we expected would be an alpha-emitter. We made a strong sample (11 millicuries) of the 2.3-day activity by chemical extraction from 500 grams of irradiated uranyl nitrate, and looked for alpha-particle activity in this sample. This experiment was a failure, probably because our sample was too thick so that alpha-particles could not get out, and our published conclusion concerning the limiting value of the half-life of the resulting alpha activity was the only serious error in our published note*.

After Abelson's rather industrious «vacation» was over I returned to the search for the alpha-particles. The lanthanum fluoride precipitate bearing the products of the decay of the strong element 93 sample did show an alpha-activity, which was at first suspected of being due to contamination with natural uranium. However a measurement of the range showed that it was too long for that and therefore might actually be from the long-sought element 94. I then tried bombarding uranium directly with the 16-MeV deuterons from the cyclotron, in the hope that this might produce a different isotope of element 94 with a shorter life, giving a greater rate of alpha-particle emission. This hope proved to be well founded, and a considerably stronger alpha-activity was found in the decay products of the element 93 separated from the deuteron-bombarded sample. The next slide (Fig. 6) shows the nuclear reactions involved in the formation of the two isotopes of element 94. At the time of which I am speaking, the half-lives of the alpha emitters and the correct isotopic assignment of the one produced by deuterons were not known. However the names «Neptunium» and «Plutonium» had already been suggested so it seems appropriate that the corresponding chemical symbols are shown on the slide.

With these alpha-emitting samples I tried some chemical separations, finding that the alpha-activity did not belong to an isotope of protactinium, uranium, or neptunium. It was precipitated along with four-valent cerium as the iodate, indicating a similarity to thorium and four-valent uranium.

$$
\begin{aligned}
^{238}\mathrm{U} + \mathrm{n} &\rightarrow \gamma + {}^{239}\mathrm{U} \text{ (instantaneous)} \\
^{239}\mathrm{U} &\rightarrow \beta^- + {}^{239}\mathrm{Np} \text{ (23 minutes)} \\
^{239}\mathrm{Np} &\rightarrow \beta^- + {}^{239}\mathrm{Pu} \text{ (2.3 days)} \\
^{239}\mathrm{Pu} &\rightarrow \alpha + {}^{235}\mathrm{U} \text{ (24,000 years)} \\
^{238}\mathrm{U} + \mathrm{d} &\rightarrow 2\mathrm{n} + {}^{238}\mathrm{Np} \text{ (instantaneous)} \\
^{238}\mathrm{Np} &\rightarrow \beta^- + {}^{238}\mathrm{Pu} \text{ (2.1 days)} \\
^{238}\mathrm{Pu} &\rightarrow \alpha + {}^{234}\mathrm{U} \text{ (92 years)}
\end{aligned}
$$

Fig. 6. Nuclear reactions concerned in the production and decay of two plutonium isotopes. The times on the right are the half-lives of the processes indicated.

* Another error in that note (Ref. 11 in Seaborg and Segrè) is in the value given for the upper limit of the beta energy of Np-239. The value printed (0.47 MeV) is a clerical error; the observed end-point of the absorption curve in aluminum was 250 milligrams per square centimeter, corresponding to an energy of 0.7 MeV. This is in agreement with later values obtained by the same method.

The natural supposition was that plutonium followed chemically the properties of uranium and neptunium, with the lower state still more stable than it is in neptunium. However I did not participate in the final proof of this, since I left Berkeley in November 1940, to take part in the development of radar for national defense. The rest of the story belongs to Seaborg*, who took up the work after my departure, and I shall let him tell it in his own way.

* I have not given a list of references; such a list can be found in an excellent review of the subject: *The Trans-Uranium Elements*, by G. T. Seaborg and E. Segrè, *Nature*, 159 (1947) 863.

Biography

Edwin Mattison McMillan was born on 18th September, 1907, at Redondo Beach, California. He is the son of Dr. Edwin Harbaugh McMillan, a physician, and his wife, Anne Marie McMillan, *née* Mattison, who both came from the State of Maryland and were both of English and Scottish descent. The boy spent his early years in Pasadena, California, and obtained his education in that state.

McMillan attended the California Institute of Technology, obtaining a B.Sc. degree in 1928, and taking his M.Sc. degree a year later, then transferring to Princeton University for Ph.D. in 1932. The same year he entered the University of California at Berkeley as a National Research Fellow. The thesis he submitted for Ph.D. was in the field of molecular beams, and the problem he undertook as a National Research Fellow was the measurement of the magnetic moment of the proton by a molecular beam method. After two years on this work and one as a research associate he became a Staff Member of the Radiation Laboratory under Professor E. O. Lawrence, studying nuclear reactions and their products, and helping in the design and construction of cyclotrons and other equipment, and a member of the Faculty in the Department of Physics at Berkely, being appointed Instructor in 1935, Assistant Professor in 1936, Associate Professor, 1941, and Professor in 1946.

During the Second World War, McMillan was on leave from November, 1940, to September, 1945, engaged on national defence research, serving (1940–1941) in the Radiation Laboratory, Massachusetts Institute of Technology; (1941–1942) U. S. Navy Radio and Sound Laboratory, San Diego; (1942–1945) Manhattan District, Los Alamos.

It was during 1945 that he had the idea of «phase stability» which led to the development of the synchroton and synchro-cyclotron; these machines have already extended the energies of artificially accelerated particles into the region of hundreds of MeV and have made possible many important researches.

McMillan returned to the University of California Radiation Laboratory

as Associate Director from 1954–1958, when he was raised to Deputy Director and finally Director, in the same year.

In 1951 he received the 1950 Research Corporation Scientific Award, and in 1963 he shared the Atoms for Peace Award with Professor V. I. Veksler.

Professor McMillan is a Fellow of the American Physical Society and the American Academy of Arts and Sciences, a member of the National Academy of Sciences and the American Philosophical Society, and from 1954–1958 he served on the General Advisory Committee to the Atomic Energy Commission. In 1960 he was appointed to the Commission on High Energy Physics of the International Union of Pure and Applied Physics.

An honorary doctorate in science was awarded to him by the Rensselaer Polytechnic Institute in 1961, and by Gustavus Adolphus College in 1963.

While serving in the Faculty of Physics at Berkeley, McMillan married Elsie Walford Blumer, a daughter of Dr. George Blumer, Dean Emeritus of the Yale Medical School. There are three children of the marriage – Ann Bradford (1943), David Mattison (1945) and Stephen Walker (1949).

Glenn T. Seaborg

The transuranium elements: present status

Nobel Lecture, December 12, 1951

Mina Damer och Herrar.

Jag är inte van vid kemiska ord på svenska, och det är för att mina föräldrar aldrig talade kemi i hemmet. Jag skulle gärna ändå velat hålla hela denna föreläsning på svenska. Men–om jag talade svenska så skulle mina vänner McMillan och Lawrence inte förstå mer än hälften av vad jag säger. Jag ska därför tala engelska, och jag vet att ni alla förstår det språket mycket bra.

As I continue, speaking in English, I want to assure my British and American colleagues here, Sir John Cockcroft and Professors Walton, Lawrence, and McMillan, that they haven't missed anything yet. I want to begin by saying that I am fortunate in being a member of a Chemistry Department which has a long standing tradition of great breadth of research interests, leading to an early program in nuclear chemistry under the active leadership of men like G. N. Lewis and W. M. Latimer. The work on the transuranium elements which I am about to describe was made possible by this circumstance and because of the unusual and excellent spirit of cooperation which exists at the Radiation Laboratory, under the direction of Professor E. O. Lawrence, where the cyclotron bombardments so crucial to the final results were made. I shall try in the course of my lecture to point out the contributions to this work of my many colleagues but unfortunately it will not be possible to do this adequately in the time available.

Beginning at essentially the point where Professor McMillan left off in his lecture, I shall first describe our work on plutonium. The beginning of this can be considered to have taken place in the fall of 1940 when I asked a graduate student, Arthur C. Wahl, to consider the possibility of studying the tracer chemical properties of element 93 as a thesis problem, a suggestion which he was happy to accept. This, and related work on element 94, was carried on in collaboration with Dr. Joseph W. Kennedy who, like myself, was at that time an instructor in the Department of Chemistry at the University of California. After Dr. McMillan's departure from Berkeley in November, 1940, and his gracious assent to our continuation of the search for, and

possible identification of element 94, our group turned its major efforts to this problem.

A bombardment of uranium oxide with the 16-MeV deuterons from the 60-inch cyclotron was performed in December, 1940. Alpha radioactivity was found to grow into the chemically separated element 93 fraction, and this alpha-activity was chemically separated from the neighboring elements, especially elements 90 to 93 inclusive, in experiments performed during the following months. These experiments, which constituted the positive identification[1,2] of element 94, showed that this element has at least two oxidation states, distinguishable by their precipitation chemistry, and that it requires stronger oxidizing agents to oxidize element 94 to the upper state than is the case for element 93. The particular isotope identified has been shown to be of mass number 238 and the reactions for its preparation are shown in the first slide (Fig. 1).

$$^{238}_{92}\mathrm{U} + {}^{2}_{1}\mathrm{H} \longrightarrow {}^{238}_{93}\mathrm{Np} + 2\mathrm{n}$$

$$^{238}_{93}\mathrm{Np} \xrightarrow[2.1\ \mathrm{days}]{\beta^-} {}^{238}_{94}\mathrm{Pu}\ (90\ \mathrm{years},\ \alpha)$$

Fig. 1.

The chemical properties of elements 93 and 94 were studied by the tracer method at the University of California for the next year and a half. These first two transuranium elements were referred to by our group simply as «element 93» and «element 94» until the spring of 1942, at which time the first detailed reports concerning these elements were written[3,4,5]. In order to write the report[4] on the chemical properties, it became necessary to have chemical symbols for the two elements. It was remembered that McMillan had suggested the name «neptunium» (symbol Np) for element 93 after Neptune, the planet immediately beyond Uranus, which gives its name to uranium, and, therefore, it was thought proper that element 94 should assume the name «plutonium» (symbol Pu) after the next planet Pluto. These names and symbols have been adopted officially by the International Union of Chemistry and will be used here.

The isotope of plutonium which is of major importance is the one with mass number 239. The search for this isotope, as a decay product of ^{239}Np, was going on almost simultaneously, and these experiments were being carried on by the same group with the added collaboration of Dr. E. Segrè. The isotope ^{239}Pu was identified and its possibilities as a nuclear energy

source were established[6] during the spring of 1941 using a sample prepared by the decay of cyclotron-produced ^{239}Np and later purified by taking advantage of the then known chemistry of plutonium.

Once the value of the isotope ^{239}Pu was thus established, the paramount problem was that of producing it on a large scale and isolating it after production. The production problem was solved through the development of the chain reacting units, or piles, utilizing the neutron-induced fission reaction on ^{235}U in natural uranium, in which the extra neutrons beyond those needed to perpetuate the chain reaction are absorbed by ^{238}U to form the desired isotope ^{239}Pu. These well-known reactions are summarized in the following slide (Fig. 2).

$$^{235}U + n \rightarrow \text{fission products} + \text{energy} + \text{neutrons}$$

$$^{238}U + n \rightarrow {}^{239}_{92}U \xrightarrow[23.5\ \text{min}]{\beta^-} {}^{239}_{93}Np \xrightarrow[2.33\ \text{days}]{} {}^{239}_{94}Pu$$

Fig. 2.

The isolation of the plutonium was to be done by chemical means, and in the spring of 1942, I and a number of my colleagues moved to the Metallurgical Laboratory at the University of Chicago to work on this problem. Among the people who made outstanding contributions to this separation program, both at Chicago and at other sites, was Dr. I. Perlman, who, it is interesting to note, before this time had made an outstanding reputation in another field, the application of radioactive tracers to physiology and biochemistry. Investigations continued at the University of California under the direction of W. M. Latimer and A. C. Wahl, leading to further discoveries of importance to the program.

During the first months at the Metallurgical Laboratory, intensive effort was directed toward defining the process which was to be used in the production plants which were then being planned. Although it was felt that the separation process would depend on the use of the two oxidation states of plutonium which had been discovered during the early work at the University of California, the actual details, such as the best carrier compounds and best oxidizing and reducing agents, had not yet been discovered. Dr. S. G. Thompson is largely responsible for the conception and early development of the process which was finally chosen. I cannot, of course, give recognition individually to the large group of scientists who participated so successfully in the diverse phases of this over-all problem. In addition, although this

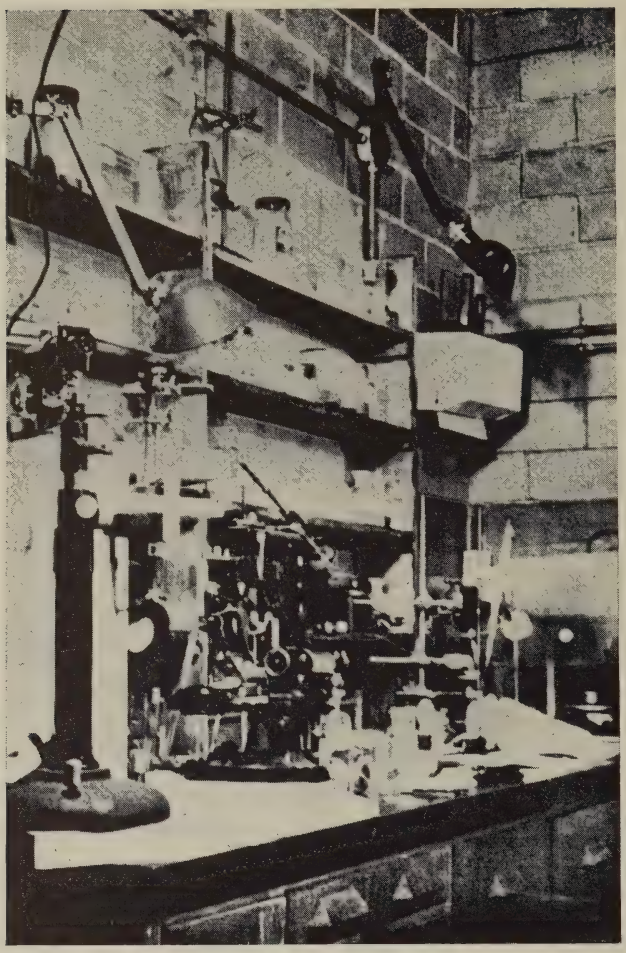

Fig. 3. First laboratory for the study of pure plutonium, Metallurgical Laboratory, Chicago, 1942.

lecture is concerned primarily with the basic chemistry and physics of the transuranium elements, mention should be made of the outstanding contribution of the chemists and engineers of the du Pont Company in the adaptation of the plutonium process for industrial operation and in the plant design, construction and operation.

The laboratory investigations were conducted on the tracer scale, and it soon became apparent that tests would be necessary under conditions where the plutonium concentration would be the same as that anticipated in the production plant. This proved a great difficulty since no one had actually

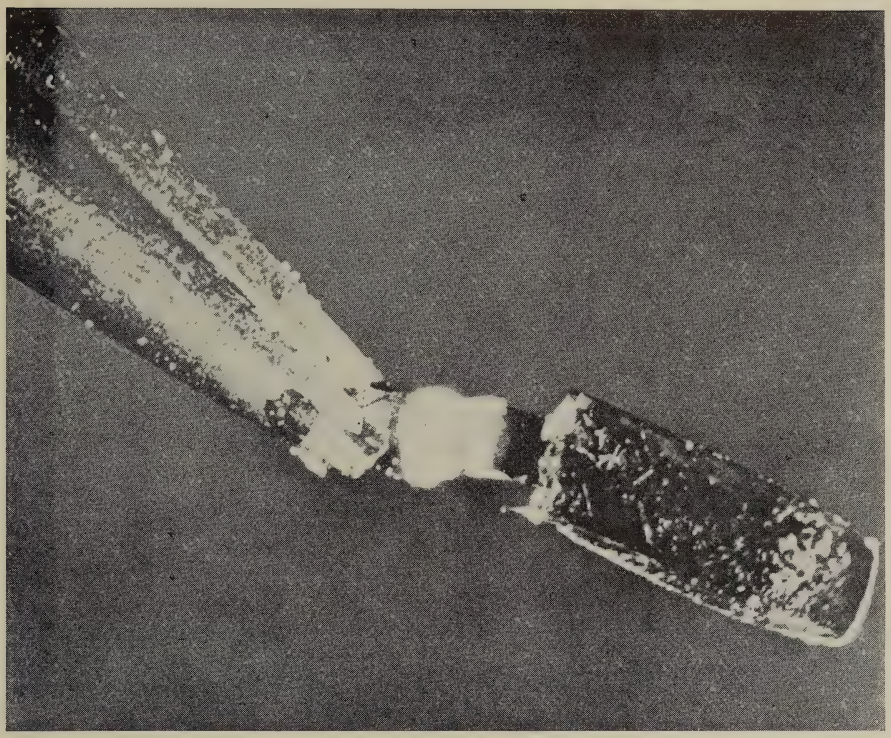

Fig. 4. This highly magnified plutonium compound is the first to be weighed by man and is here shown on a platinum shovel held by forceps. (Magnified approximately 13-fold.) (*Courtesy of Life Magazine.*)

seen any plutonium up to this time, and the design of the separation plants had to begin immediately so that the construction of these plants could take place at the same time as the construction of the chain reacting units. This problem was solved by producing an actually weighable amount of plutonium by bombarding large amounts of uranium with neutrons from the cyclotron, the first time a weighable amount of any transmutation product had been produced with a particle acceleration machine, and by working with this material in such extremely small volumes that ordinary concentrations would prevail. The field which embraces the study of chemical material on this, the microgram scale of operation, has been given the name of «ultramicrochemistry» by P. L. Kirk, a pioneer investigator in the field of quantitative chemistry on the microgram scale.

This work was carried on chiefly by Dr. B. B. Cunningham and L. B. Werner, who had previously been working in the biochemical field, and the

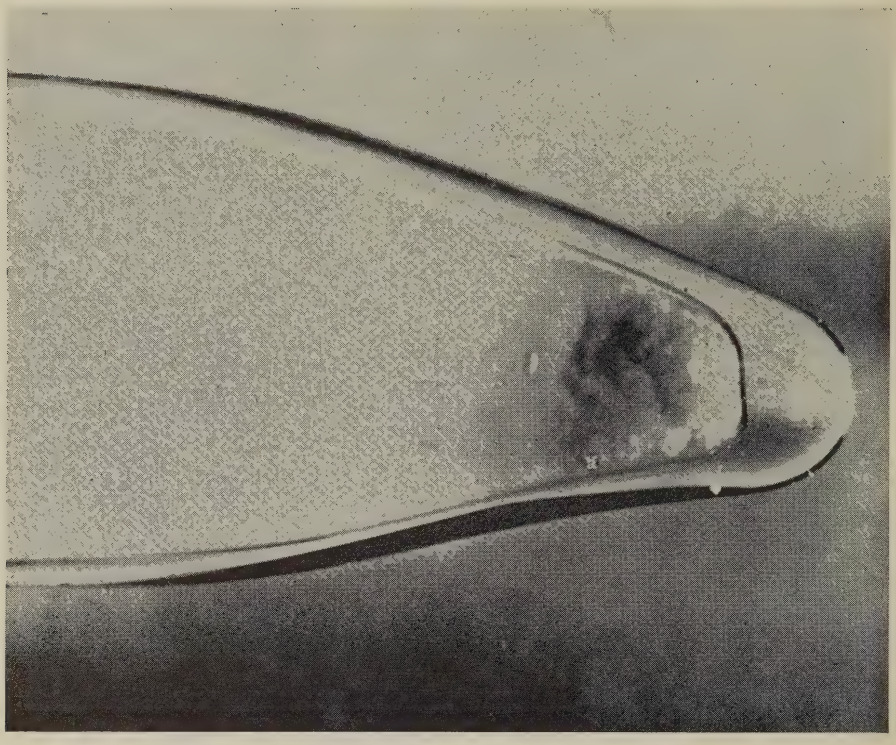

Fig. 5. Twenty micrograms of pure plutonium hydroxide in capillary tube (magnified about 13-fold) isolated in September, 1942. The plutonium is the cloud-like mass at the bottom of the capillary «test tube».

next slide (Fig. 3) shows a photograph of the room at the Metallurgical Laboratory where they worked. The small amount of space in which they worked, apparent from the photograph, is all the more remarkable when one considers that Werner is a man who is more than two meters tall. The first pure chemical compound[7] of plutonium free from carrier material and all other foreign matter was prepared by Cunningham and Werner on August 18, 1942, and the first weighing of such a compound occurred on September 10, 1942, when 2.77 micrograms of the oxide was weighed. A picture of this oxide is shown on the following slide (Fig. 4). Not only was it possible to test the separation processes at the actual concentrations which were going to be used in the plant, tests which were of inestimable importance, but a great number of pure chemical compounds of plutonium were produced and its chemical properties were well studied by a number of investigators working on the ultramicrochemical scale. The next slide shows

an early photograph of a compound of plutonium (Fig. 5). Since plutonium has become available on a production scale from the chain reacting units the chemical properties have been thoroughly investigated until they are as well-known as those of numerous elements that have been studied for a great many years. A contribution of importance was made by W. H. Zachariasen of the University of Chicago, who was able to use his X-ray technique to identify or help identify a number of the compounds which were synthesized, and in many cases, thus to identify their structure.

Before proceeding with a discussion of the other transuranium elements, I should like to say a few more words about neptunium. Another isotope, ^{237}Np, was discovered early in 1942 in collaboration with Wahl[8]. This isotope is the decay product of the previously[9,10] known approximately 7-day beta particle emitting ^{237}U which is formed as the result of an n, 2n reaction on ^{238}U. The reactions for its production are shown in the next slide (Fig. 6).

$$^{238}U + n \longrightarrow {}^{237}U + 2n$$

$$^{237}_{92}U \xrightarrow[6.6\ \text{days}]{\beta^-} {}^{237}_{93}Np\ (2.20 \times 10^6\ \text{years}, \alpha)$$

Fig. 6.

This isotope is of particular importance because it has a very long half-life and because it is available in weighable amounts. The first pure neptunium in the form of compounds of the isotope ^{237}Np was isolated by L. B. Magnusson and T. J. La Chapelle[11] at the wartime Metallurgical Laboratory in October, 1944, and a picture is shown in the following slide (Fig. 7). It is fortunately produced as a by-product in the chain reacting piles which has led to the isolation of gram amounts for research purposes. The chemical properties of neptunium in the macroscopic state have been studied with such material, and this has led to a thorough knowledge of the chemistry of this element.

After the completion of the most essential part of the investigations concerned with the chemical processes involved in the production of plutonium at the wartime Metallurgical Laboratory, our attention turned to the problem of synthesizing and identifying the next transuranium elements. As collaborators in this endeavor, I turned to Albert Ghiorso, trained in electrical engineering, who played a dominant rôle in the development of the electronic instruments which were used in our radioactivity investigations, and to two young chemists, R. A. James and L. O. Morgan, who had been

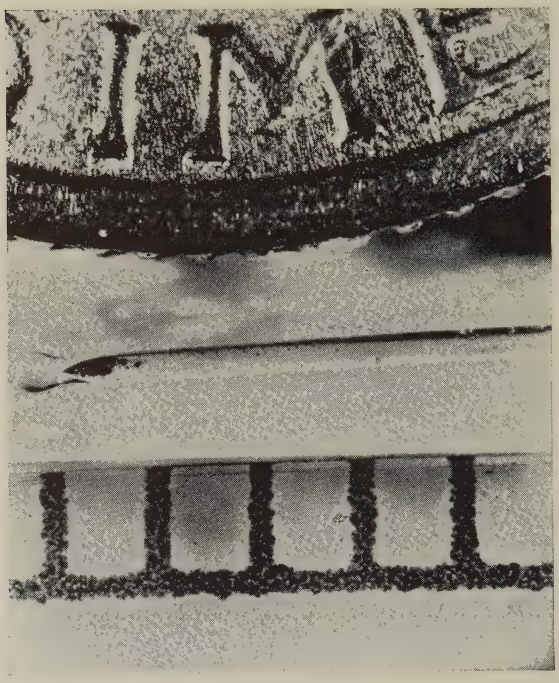

Fig. 7. Ten micrograms of pure neptunium oxide at bottom of capillary tube (magnification about 10-fold) isolated in 1944. Above the sample is a U.S. ten-cent piece (dime) and below is a millimeter scale.

especially proficient in the investigations involving the chemistry of the transuranium elements. These investigations, supplemented by later work at the University of California, served as the thesis material for James and Morgan in connection with the graduate work which they undertook at the University a couple of years later.

There followed quite a period during which the attempts to synthesize and identify elements 95 and 96 bore no fruit. These unsuccessful experiments were based on the premise that these elements should be much like plutonium in that it should be possible to oxidize them to the (VI) oxidation state and utilize this in the chemical isolation procedures. It was not until the middle of the summer of 1944, upon the first recognition that these elements were part of an actinide transition series, about which I will say more later, that any advance was made, and then progress came quickly.

As soon as it was recognized that these elements should be oxidized above the (III) state only with extreme difficulty, if at all, the identification of an

isotope of element 96 followed immediately. Thus the isotope ^{242}Cm was identified[12] in the summer of 1944 as a result of the bombardment of ^{239}Pu with 32 MeV helium ions in the Berkeley 60-inch cyclotron. The reaction involved was ^{239}Pu (α, n)^{242}Cm, and the bombardment took place in the Berkeley 60-inch cyclotron after which the material was shipped to the Metallurgical Laboratory for chemical identification. I should like to digress for a moment here to point out that our research program owes much to the efforts of Dr. J. G. Hamilton, T. M. Putnam, Jr., G. B. Rossi, and their collaborators who have operated the 60-inch cyclotron and made the bombardments which led to the discoveries of elements 96, and elements 97 and 98 to be mentioned later. In fact, I should like to pause further and recall at this time that five of the six presently known transuranium elements were first identified as a result of their initial production through the use of the Berkeley 60-inch cyclotron.

The identification[13,14] of element 95 followed soon thereafter. This came in the very late fall of 1944 as a result of the bombardment of ^{239}Pu with pile neutrons, the production reactions being as shown in the following slide (Fig. 8).

$$^{239}\mathrm{Pu} + \mathrm{n} \rightarrow {}^{240}\mathrm{Pu} + \gamma$$
$$^{240}\mathrm{Pu} + \mathrm{n} \rightarrow {}^{241}\mathrm{Pu} + \gamma$$
$$^{241}_{94}\mathrm{Pu}\ (14\ \text{years}) \xrightarrow{\beta^-} {}^{241}_{95}\mathrm{Am}\ (475\ \text{years}, \alpha)$$

Fig. 8.

At the same time the isotope ^{242}Cm is formed as shown in the following slide (Fig. 9), and this is presently the best method for its production.

$$^{241}\mathrm{Am} + \mathrm{n} \xrightarrow{\beta^-} {}^{242}\mathrm{Am} + \gamma$$
$$^{242}_{95}\mathrm{Am} \begin{pmatrix} 16\ \text{hours upper state} \\ 100\ \text{years lower state} \end{pmatrix} \xrightarrow{\beta^-} {}^{242}_{96}\mathrm{Cm}\ (162\ \text{days}, \alpha)$$

Fig. 9.

There are a couple of comments which should be made here concerning the rare-earthlike properties of these two elements. Our hypothesis that they should have a stable (III) oxidation state and greatly resemble the rare earth elements in their chemical properties proved to be so true that for a time it

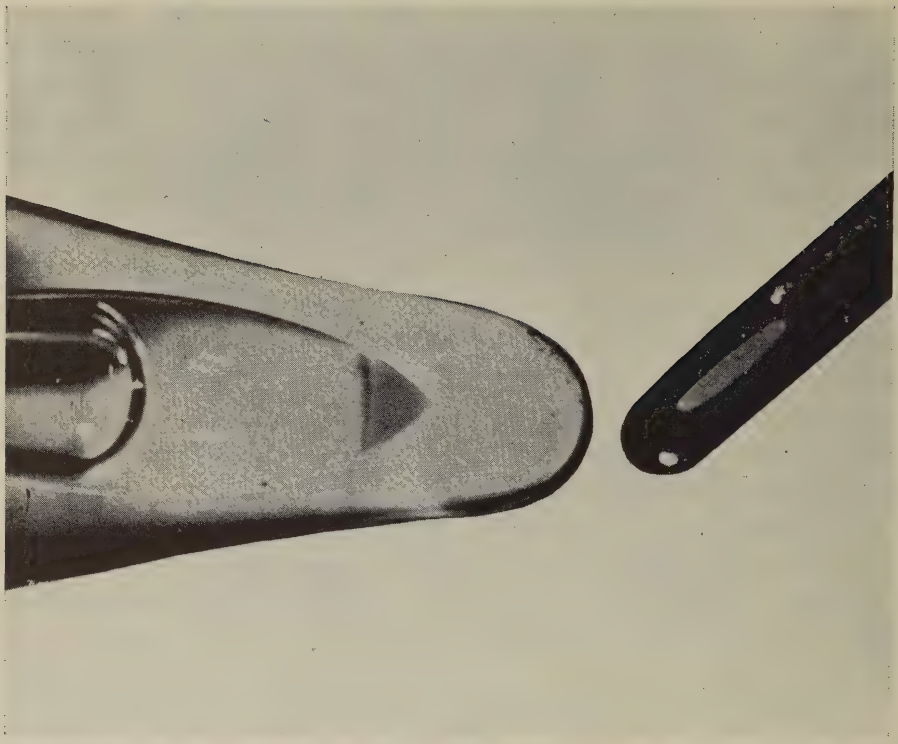

Fig. 10. First americium compound (hydroxide) in bottom of tube (*centre*). Isolated in the fall of 1945. Eye of sewing needle below indicates magnification of about 10-fold.

appeared to be most unfortunate. The better part of a year was spent in trying to separate chemically the two elements from each other and from the rare earth elements but without success, and although we felt entirely confident, on the basis of their radioactive properties and the methods of production, that isotopes of elements 95 and 96 had been produced, the chemical proof remained to be demonstrated. The elements remained unnamed during this period of futile attempts at separation, although one of our group insisted in referring to them by the names « pandemonium » and « delirium » in recognition of our difficulties. However, they were finally separated and completely identified chemically, and, in fact, their present names were eventually proposed on the basis of their chemical properties but with a more serious basis. The name « americium » (symbol Am) was suggested for element 95, thus naming it after the Americas by analogy with the naming of its rare-earth homologue europium after Europe, and the name « curium » (symbol Cm) was suggested for element 96 after Pierre and Marie Curie

Fig. 11. Photograph of curium hydroxide in bottom of microcentrifuge cone. (Magnified about 2-fold.)

by analogy with the naming of its homologue gadolinium after Gadolin. These names and symbols will be used here and were used in the preceding slides.

Americium was first isolated by Cunningham[15] in the form of a pure compound in the fall of 1945 at the wartime Metallurgical Laboratory, and a photograph is shown on the next slide (Fig. 10). It can be prepared in milligram amounts by the neutron bombardment of plutonium according to reactions shown on a foregoing slide (Fig. 8), and thus it has been possible to investigate its chemical properties extensively through the use of macroscopic quantities.

Curium was first isolated in the form of a pure compound of ^{242}Cm by L. B. Werner and I. Perlman[16] at the University of California during the fall of 1947. A photograph is shown in the following slide (Fig. 11). The isotope ^{242}Cm is so highly radioactive, due to its short half-life, that chemical investigations with it in macroscopic concentrations are very difficult. Nevertheless

a large number of such investigations have been carried on and much has been learned about its chemical properties.

More recent work has led to the identification of a number of heavier isotopes of americium, curium, and plutonium which present interesting possibilities for the further nuclear and chemical investigation of these elements. The nuclide ^{242m}Am formed by the neutron bombardment[12,13,17] of ^{241}Am decays partially by electron capture[18] to the isotope of plutonium, ^{242}Pu. The latter nuclide[19] is an alpha emitter with the comparatively long half-life of 5×10^5 years and, therefore, may be important in future investigations of the chemical properties of plutonium since it has a specific activity some twenty times lower than that of ^{239}Pu. This material can be transmuted by neutron bombardment[20,21] to the comparatively long-lived ^{243}Am (about 10^4 years) and, therefore, following chemical separation this latter isotope can be made available comparatively free of other isotopes of americium. Similarly, such ^{243}Am can be transmuted by neutron bombardment[22,23] to the comparatively long-lived ^{244}Cm (about 10 years) which can thus be made available in a comparatively pure state following chemical separation. These reactions are summarized on the following slide (Fig. 12).

$$^{242m}_{95}\mathrm{Am} \xrightarrow{\mathrm{EC}} {}^{242}_{94}\mathrm{Pu}\ (\sim 5 \times 10^5 \text{ years}, \alpha)$$

$$^{242}\mathrm{Pu} + \mathrm{n} \rightarrow {}^{243}\mathrm{Pu} + \gamma$$

$$^{243}_{94}\mathrm{Pu} \xrightarrow[5 \text{ hours}]{\beta^-} {}^{243}_{95}\mathrm{Am}\ (\sim 10^4 \text{ years}, \alpha)$$

$$^{243}\mathrm{Am} + \mathrm{n} \rightarrow {}^{244}\mathrm{Am} + \gamma$$

$$^{244}_{95}\mathrm{Am} \xrightarrow[\sim 25 \text{ min}]{\beta^-} {}^{244}_{96}\mathrm{Cm}\ (\sim 10 \text{ years}, \alpha)$$

Fig. 12.

Thus the isotope ^{243}Am, with a specific activity some twenty times lower than that of ^{241}Am, may supplant ^{241}Am for use in the chemical studies of the future; it is not yet known whether ^{243}Am is beta stable, but if it is not, its partial half-life for beta emission appears to be longer than 10^4 years so that these considerations would not be altered. Due to the difficulties in studying the chemical properties of curium through the use of the isotope ^{242}Cm, the isotope ^{244}Cm with twenty times lower specific activity produced as just indicated, offers great hope for the simplification of future chemical studies of curium.

After the return at the conclusion of the war of a number of us to the University of California late in 1945 and early 1946, a part of the effort was put into the problem of the possible production and identification of further transuranium elements. S. G. Thompson, who had done so much in connection with the development of the separation processes for plutonium during the war, undertook to collaborate on the search for element 97 as the basis for his thesis research problem, and the further collaboration of Albert Ghiorso was also sought. Many problems had to be solved, mostly in connection with the small amounts of material available for irradiation and the intense radioactivity of this material. To help counterbalance the many difficulties were the advances which had been made in the possibility of predicting the radioactive properties of the expected isotopes and especially the exact predictions of the chemical properties made possible on the basis of the actinide concept. According to this view, the elution positions of the transcurium elements should be analogous to those of the transgadolinium elements in the ion-exchange column separation method. This knowledge proved of inestimable benefit in planning the ultimately successful experiments. Two approaches to the problem were undertaken: namely, that of neutron irradiation in the chain reacting piles and that of charged particle bombardment in the 60-inch cyclotron. The difficulties attending the neutron irradiation are illustrated in the following slide (Fig. 13).

$$^{244}\mathrm{Cm}\,(\mathrm{n},\gamma)\,^{245}\mathrm{Cm}\,(\mathrm{n},\gamma)\,\ldots\ldots\,^{249}\mathrm{Cm}$$

$$^{249}_{96}\mathrm{Cm} \xrightarrow{\beta^-} {}^{249}_{97}\mathrm{Bk}$$

$$^{249}\mathrm{Bk}\,(\mathrm{n},\gamma)\,^{250}\mathrm{Bk}$$

$$^{250}_{97}\mathrm{Bk} \xrightarrow{\beta^-} {}^{250}_{98}\mathrm{Cf}$$

Fig. 13.

The prediction that curium must have a mass number as high as 249 before a negative beta particle emitter is reached is based on considerations involving the nuclear energy surface and the systematics of radioactivity which cannot be described here because of the lack of time. These considerations are, of course, not exact and it is quite possible that negative beta particle emission will be reached as early as ^{247}Cm. The considerations are negated if there should exist a pronounced closed subshell at 148 neutrons in which case the isotopes of curium above ^{244}Cm might be quite short-lived.

Whatever the reason for it may be, the neutron irradiations did not yield

positive results, and, therefore, I will go on to describe the cyclotron irradiations by which it was finally possible in December, 1949 to identify[24] an isotope of the element with atomic number 97 as a result of the irradiation of ^{241}Am with 35 MeV helium ions. This was followed within a month by the identification[25] of an isotope of element 98 through the irradiation of ^{242}Cm with 35 MeV helium ions, in a research carried on by the same investigators with the addition of a young colleague, K. Street, Jr., who had already contributed very much to our knowledge of the chemical properties of the transuranium elements. The first identification experiment in the case of element 98 involved the production and separation of only a few thousand atoms. The reactions involved are summarized in the following slide (see Fig. 14).

$$^{241}_{95}\mathrm{Am} + {}^{4}_{2}\mathrm{He} \rightarrow {}^{243}_{97}\mathrm{Bk} \quad (4.6 \text{ hours, } K/\alpha \cong 10^{3}) + 2n$$

$$^{242}_{96}\mathrm{Cm} + {}^{4}_{2}\mathrm{He} \rightarrow {}^{244}_{98}\mathrm{Cf} \quad (45 \text{ min, } \alpha) + 2n$$

Fig. 14.

It should be mentioned that the successful handling in a safe manner of the huge amounts of radioactivity in the target material was made possible through the use of the excellent protective equipment provided by Nelson Garden and the members of his Health Chemistry Group at our Laboratory.

The name «berkelium» (symbol Bk) was suggested for element 97 after the city where the work was done, in analogy with the naming of its chemical homologue terbium, which was named after the village of Ytterby here in Sweden where the rare earth minerals were first found. The name «californium» (symbol Cf) was suggested for element 98 in honor of the university and state where the work was done; thus the name does not reflect its strong homology to dysprosium. These names are used in the present talk including the various slides.

Soon following this work, it became possible on the basis of the interesting pioneer carbon-ion bombardment experiments of Miller, Hamilton, Putnam, Haymond, and Rossi[26] in the 60-inch cyclotron to produce isotopes of californium in better yield by this method. These experiments, in which uranium is bombarded with carbon ions, led to the production[27] of califor-

nium isotopes according to the reactions given in the following slide (see Fig. 15).

$$^{238}_{92}\mathrm{U} + {}^{12}_{6}\mathrm{C} \rightarrow {}^{244}_{98}\mathrm{Cf} \quad (45\ \text{min}, \alpha) \quad + 6\mathrm{n}$$

$$^{238}_{92}\mathrm{U} + {}^{12}_{6}\mathrm{C} \rightarrow {}^{246}_{98}\mathrm{Cf} \quad (36\ \text{hours}, \alpha) \quad + 4\mathrm{n}$$

Fig. 15.

There are, of course, many more transuranium nuclides known that I have not been able to describe in the time available. For the sake of completeness these are summarized in the following slide (Fig. 16).

It should be emphasized that these nuclides are the product of the investigations of a number of laboratory groups besides our own. I should like to mention especially the work of W. M. Manning, M. H. Studier and coworkers of the Argonne National Laboratory in Chicago, Illinois, as well as the work of the United States Atomic Energy Commission's Los Alamos Laboratory, and the Chalk River Laboratory of the Canadian National Research Council. I feel that I should especially single out the contributions of my colleague Albert Ghiorso whose name, as you may have noticed, has come up time after time as I have gone through my description here of the work on the transuranium elements. Ghiorso has been a key man throughout and is one of those individuals without whom a laboratory such as ours simply could not run.

I suppose that I should say a few words with respect to the possibility for future production and identification of additional transuranium elements, especially in view of the possibility of their production by heavy-ion bombardment of transuranium elements. As an aid to such a program the radioactive properties can be estimated, as I have already indicated, on the assumption of a smooth nuclear energy surface and the systematics of radioactivity. Again, I must emphasize that such considerations are negated in the event that a stable subshell of 148 neutrons should be found to exist, and this must be regarded as a definite possibility. It is interesting to note that our considerations on the systematics of spontaneous fission[28] indicate that this method of decay will not compete seriously with radioactive decay until the region just beyond element 100. The following slide (Fig. 17) shows a number of predicted half-lives for radioactive decay.

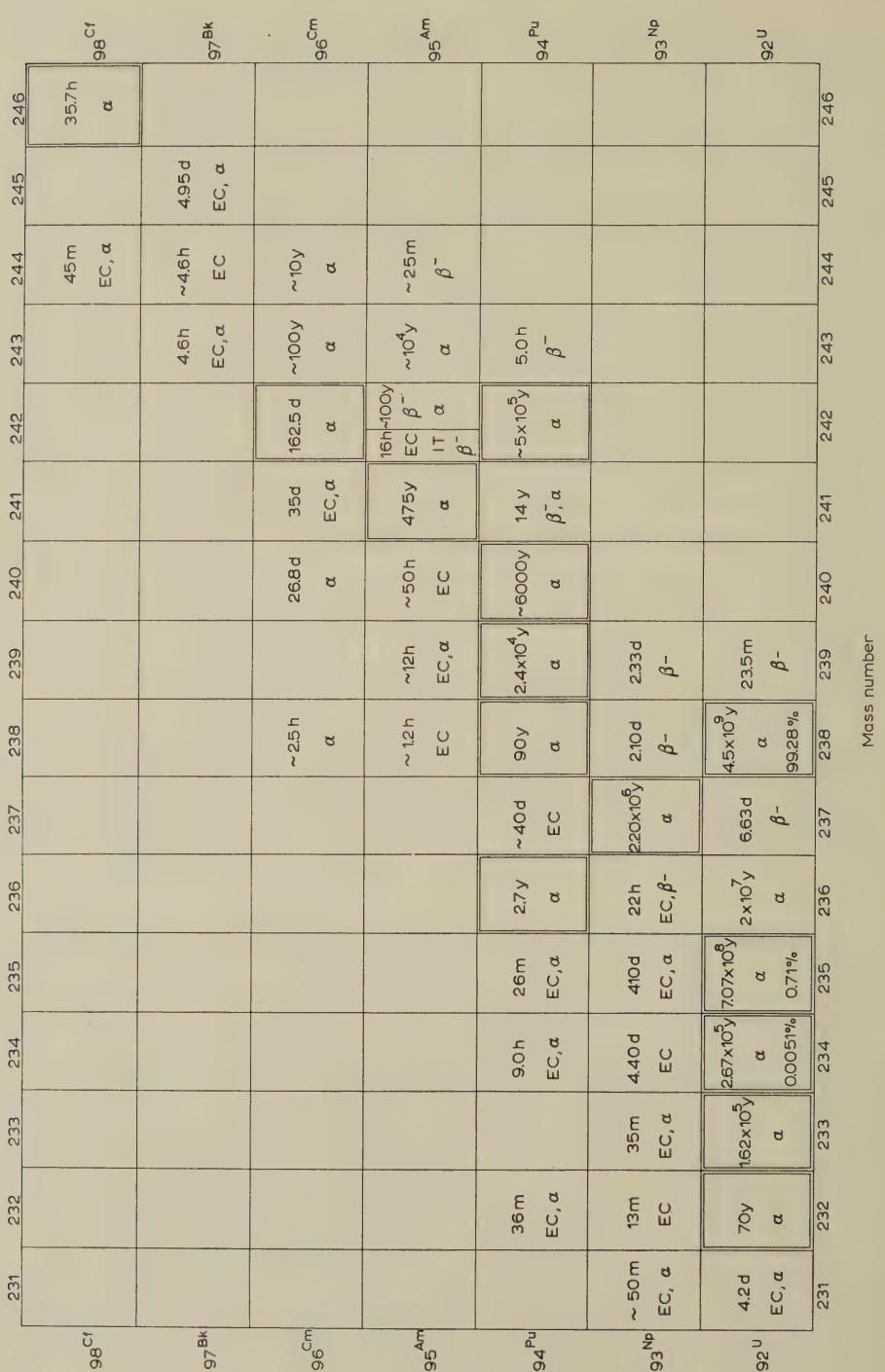

Fig. 16. Summary of transuranium nuclides.

Predicted Half-Lives

^{249}Bk	α	years
^{252}Cf	α	years
^{249}99	EC, α	minutes
^{253}99	α	months
^{250}100	EC, α	minutes
^{252}100	α	hours
^{254}100	α	days
^{257}101	EC, α	minutes
^{258}102	α	minutes

Fig. 17.

These considerations illustrate clearly that one of the problems is that of conceiving means for producing nuclides of sufficiently high mass numbers with half-lives long enough for chemical identification. Thus, the serious problem is again the paucity of starting materials.

A hopeful aspect to the problem of the production and the identification of transcalifornium elements is the feeling that the chemical properties can be predicted with confidence. Thus it is expected that the (III) oxidation state will predominate for the next few elements. Of especial importance in planning experiments is the confidence that the elution sequence will continue to follow that of the analogous rare earths as will be described in a few minutes.

Unfortunately, there is not sufficient time to say as much as I would like about the interesting chemical properties of the transuranium group of elements. As I have already mentioned, the chemical evidence indicates that it is the tripositive oxidation state which is important here and points to a beginning with actinium of an « actinide » transition series in the same sense that the rare earth « lanthanide » series begins with lanthanum[29,30]. There is, however, the important difference that the first elements in the heavy series exhibit the property of oxidation to higher states with much greater ease than is the case for the corresponding elements in the rare earth series. The most important criterion for this classification is the probable presence of seven 5f electrons (analogous to the stable gadolinium structure) in tripositive curium (element 96) rather than the presence of the first 5f electron in thorium. In fact, there might not be any such electrons in thorium with, for example, their first appearance in protactinium (two 5f electrons) or in uranium (three 5f electrons). An important aspect of these considerations is the fact that the 5f and 6d shells of the elements in this heaviest region lie so close

together that the energy necessary for the shift from one shell to the other is in some cases within the range of chemical binding energies.

The earliest element in this series which clearly exhibits the characteristic oxidation state of (III) is uranium, the third element in the series. In going up the series from uranium, each of the successive elements exhibits a more stable (III) oxidation state than the preceding element. When the elements americium (atomic number 95) and curium (atomic number 96) are reached, the (III) state is the predominant one, and, in fact, seems to be the only state of these latter elements which is thermodynamically stable in acidic aqueous solution. In the cases of berkelium (atomic number 97) and californium (atomic number 98) only tracer experiments have been done and, therefore, the information is less certain. These experiments show that berkelium exhibits stable (III) and (IV) oxidation states[31] and indicate that californium exists solely in the (III) state[32], which behavior is in entire conformity with their expected analogy with their rare earth homologues, terbium and dysprosium, respectively.

Perhaps the most striking chemical evidence as to the course taken in the filling of the 5f electron shell in this region is that offered by the work on the separation of the tripositive actinides and lanthanides by the ion-exchange column method. The use of a cation exchange resin with an eluting agent such as ammonium citrate solution, as developed on the Plutonium Project[33], has led to a satisfactory solution of the difficult problem of separating in pure form the rare earth elements from each other. The tripositive actinide elements are equally difficult to separate from each other and from the rare earths because of their great similarity to the latter. However, the analogy in behavior of the actinide and lanthanide elements here is much greater than results from a similarity in the difficulty of separation, as can be seen from the following slide (Fig. 18). The simplicity of the apparatus used is illustrated in the next slide (Fig. 19).

A remarkable analogy in the spacing can be seen between the group californium–berkelium–curium–americium and their rare earth homologues dysprosium–terbium–gadolinium–europium. The spacings here reflect the relative changes in ionic radii which determine the relative separations in the ion-exchange adsorption method. It can be seen that the same sequence of changes in the ionic radius is encountered on filling in the 5f electrons as occurs on filling the 4f shell, and, therefore, it seems quite clear that curium represents the midway point in the actinide transition series of elements in view of its position analogous to gadolinium.

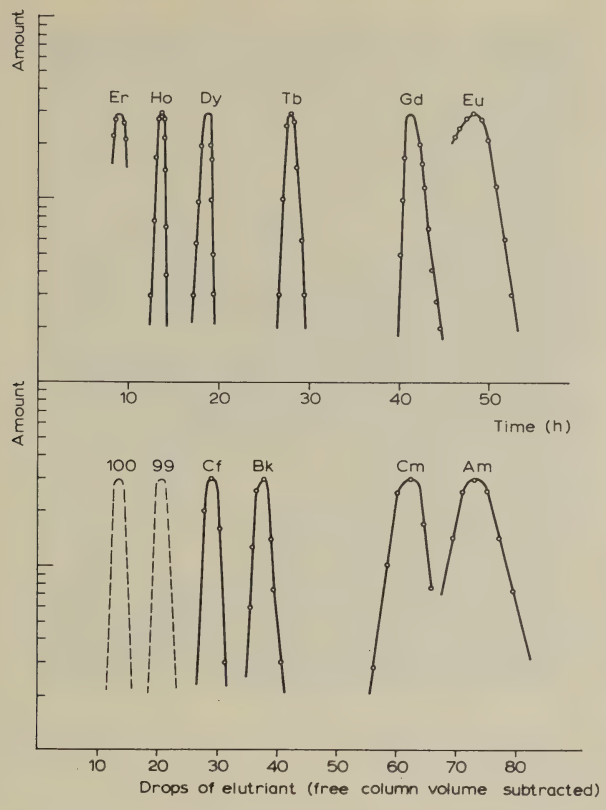

Fig. 18. Relative elution of homologous actinides and lanthanides. The data for the actinides shown here are those of K. Street, Jr., S. G. Thompson, and G. T. Seaborg[32] in which the cation exchange resin Dowex-50 was used and the elution performed at 87° C with ammonium citrate buffered with citric acid to a pH of 3.5 (total citrate concentration 0.2 *M*). The data on the lanthanide elements are those of B. H. Ketelle and G. E. Boyd[34] in which the same resin was used in a somewhat longer column and the elution performed at 100° C using a similar citrate buffer solution (pH 3.28). (The *dotted* lines show the predicted elution positions for elements 99 and 100.)

Magnetic susceptibility measurements on compounds of the heaviest elements ideally should lead to the resultant magnetic moments in fundamental units and in this way give information on the quantum states of the responsible electrons. Actually, as encountered with the rare earth elements, the situation is rather complex and the exact behavior expected for the heaviest elements, on the basis of the presence of either 5f or 6d electrons, cannot be, or at least has not been, predicted. Nevertheless, such measurements should give, and have given, some information on this point.

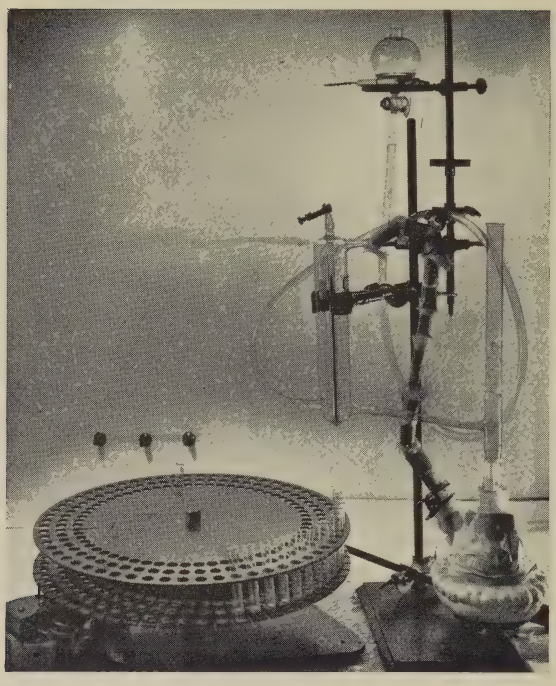

Fig. 19. Ion-exchange column with elution apparatus and automatic sample collection mechanism for separation of actinide and lanthanide elements.

The following slide (Fig. 20) shows a plot comparing the room temperature experimental magnetic susceptibilities of the lanthanide and actinide cations in such a way as to show their remarkable analogy in this property. This plot is admittedly rough because data are used from both aqueous solutions and solid compounds; these are not strictly comparable and even data from different solid compounds of the same oxidation state of an element differ somewhat due to the effects of the crystal fields and exchange effects. Nevertheless, the comparison is sufficiently reliable to show that the magnetic measurements point toward the filling of the 5f shell in the manner expected.

The next slide (Fig. 21) shows in an extremely rough and qualitative way a pictorial representation of the binding energy of the most easily removable 5f and 6d electron (of those present) for each of the heaviest elements.

The best method of presenting the actinide elements in the Periodic Table seems to be in a manner similar to that illustrated in the following slide (Fig. 22). Here are shown the fourteen elements of atomic numbers 90 to 103

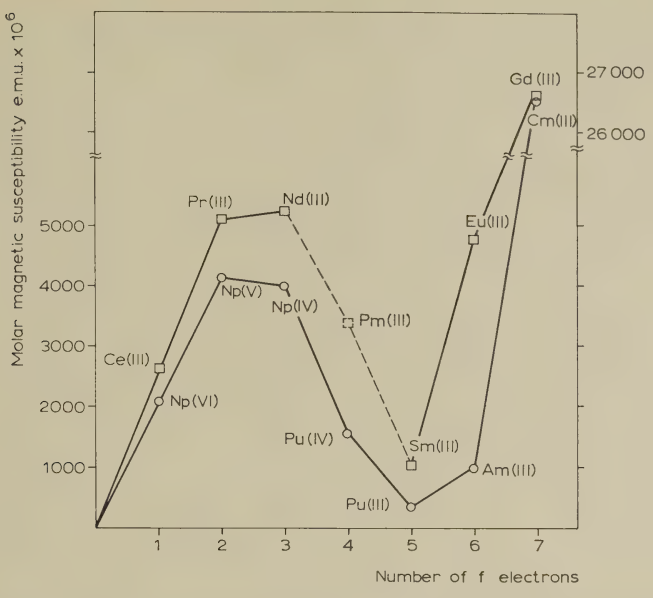

Fig. 20. Plot of molar magnetic susceptibilities of some actinide and lanthanide ions.

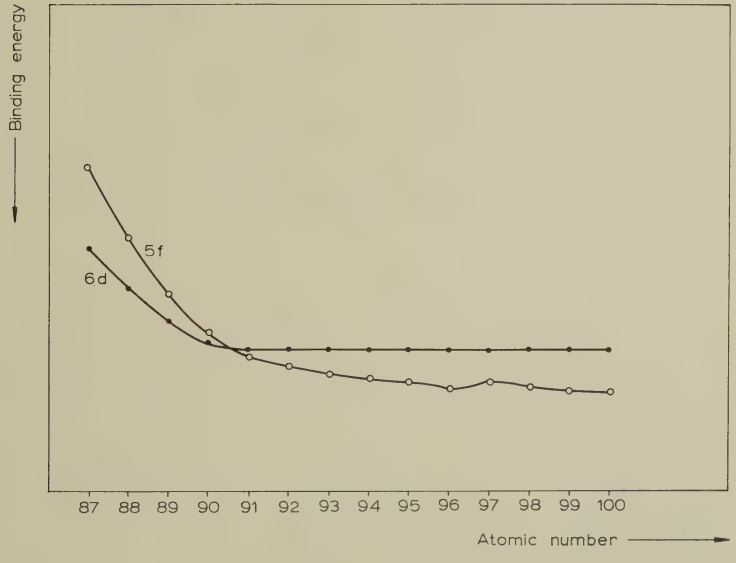

Fig. 21. Qualitative representation of electronic binding energies in the heaviest elements.

1 H 1.008																	2 He 4.003
3 Li 6.940	4 Be 9.02											5 B 10.82	6 C 12.010	7 N 14.008	8 O 16.000	9 F 19.00	10 Ne 20.183
11 Na 22.997	12 Mg 24.32											13 Al 26.97	14 Si 28.06	15 P 30.98	16 S 32.06	17 Cl 35.457	18 A 39.944
19 K 39.096	20 Ca 40.08	21 Sc 45.10	22 Ti 47.90	23 V 50.95	24 Cr 52.01	25 Mn 54.93	26 Fe 55.85	27 Co 58.94	28 Ni 58.69	29 Cu 63.57	30 Zn 65.38	31 Ga 69.72	32 Ge 72.60	33 As 74.91	34 Se 78.96	35 Br 79.916	36 Kr 83.7
37 Rb 85.48	38 Sr 87.63	39 Y 88.92	40 Zr 91.22	41 Nb 92.91	42 Mo 95.95	43 Tc	44 Ru 101.7	45 Rh 102.91	46 Pd 106.7	47 Ag 107.880	48 Cd 112.41	49 In 114.76	50 Sn 118.70	51 Sb 121.76	52 Te 127.61	53 I 126.92	54 Xe 131.3
55 Cs 132.91	56 Ba 137.36	57 La 138.92	58-71 See La series	72 Hf 178.6	73 Ta 180.88	74 W 183.92	75 Re 186.31	76 Os 190.2	77 Ir 193.1	78 Pt 195.23	79 Au 197.2	80 Hg 200.61	81 Tl 204.39	82 Pb 207.21	83 Bi 209.00	84 Po	85 At	86 Rn
87 Fr	88 Ra	89 Ac	See Ac series														

Lanthanide series	57 La 138.92	58 Ce 140.13	59 Pr 140.92	60 Nd 144.27	61 Pm	62 Sm 150.43	63 Eu 152.0	64 Gd 156.9	65 Tb 159.2	66 Dy 162.46	67 Ho 163.5	68 Er 167.2	69 Tm 169.4	70 Yb 173.04	71 Lu 174.99
Actinide series	89 Ac	90 Th 232.12	91 Pa 231	92 U 238.07	93 Np 237	94 Pu	95 Am	96 Cm	97 Bk	98 Cf					

Fig. 22. Periodic Table showing heavy elements as members of an actinide series.

inclusive, with actinium (element 89) as the prototype, listed as a series below, and in a manner similar to, the common listing of the fourteen rare earth elements of atomic numbers 58 to 71 inclusive, for which lanthanum (element 57) is the prototype. It is not proposed that this particular form of the Periodic Table has any more merit than any of a number of others which place these elements in positions homologous to the rare earth elements, since it is obvious that they can be analogously placed in a number of other types of tables or charts.

The elements 90 to 96 inclusive or the first few of them could, in addition, be listed separately below the 5d transition elements in recognition of the resemblance of the first few of these to 5d elements. This appears to be undesirable, however, since the last members of this group bear no such resemblance and it is probably impossible to draw a line as to just where the resemblance ends. It should be noted that cerium, terbium, europium, etc., could be put in two places in the Periodic Table in recognition of their two oxidation states, but this practice is not usually followed.

The group probably could have been just as well described by some other term rather than « actinide », which is derived from straight analogy with the term « lanthanide ». Irrespective of the name which usage will finally assign to this group of elements, however, it seems that the outstanding characteristics of the group, namely the « eka-gadolinium » character of curium (and the presumed « eka-lutetium » character of element 103), together with the regularly increasing trend toward actinium-like character in going from thorium to curium, are best represented by listing these elements in corresponding positions under the rare earth elements if it is desirable *to give each element only one place in the Periodic Table.*

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Biography

Glenn Theodore Seaborg was born in Ishpeming, Michigan, on April 19, 1912. At the age of 10 he moved with his family to California, in 1929 he graduated at David Starr Jordan High School in Los Angeles as valedictorian of his class.

He entered the University of California, Los Angeles, in 1929, and received the degree of Ph.D. in Chemistry from the University of California, Berkeley, in 1937. From 1937 to 1939 he was the personal laboratory assistant of the late G. N. Lewis, with whom he published a number of scientific papers. In 1939, Dr. Seaborg was appointed an instructor in chemistry at the University of California, Berkeley, where he was promoted to Assistant Professor in 1941, and to Professor of Chemistry in 1945. In 1946, he also took responsibility for direction of nuclear chemical research at the Lawrence Radiation Laboratory, operated for the Atomic Energy Commission by the University of California; from 1954 to 1961, he was Associate Director of LRL. In the same year, he was appointed by President Truman to be a member of the AEC's first General Advisory Committee, a post he held until 1950. In 1958, he was appointed Chancellor of the University of California at Berkeley. In that capacity he served until his appointment by President Kennedy to the Atomic Energy Commission in 1961, when he was designated Chairman of the Commission. His term of office expires in 1968. From 1959 to 1961, he was also a member of the President's Science Advisory Committee. Dr. Seaborg was given a leave of absence from the University of California from 1942–1946, during which period he headed the plutonium work of the Manhattan Project at the University of Chicago Metallurgical Laboratory. He was co-discoverer of plutonium and all further transuranium elements through element 102.

In addition to the discovery of transuranium elements, Dr. Seaborg and his colleagues are responsible for the identification of more than 100 isotopes of elements throughout the Periodic Table. He is also author of the actinide concept of heavy element electronic structure. In this connection, Dr. Seaborg demonstrated that the heavy elements form a « transition » series of ac-

tinide elements in a manner analogous to the rare-earth series of lanthanide elements. The concept demonstrated how the heavy elements fit into the Periodic Table and thus demonstrated their relationships to the other elements.

The body of information assembled in Dr. Seaborg's laboratory has made it possible to predict the radioactive characteristics of many isotopes of elements still to be found. Under Dr. Seaborg's leadership, also, whole new bodies of methodology and instrumentation have been developed and have become a cornerstone of modern nuclear chemistry. Dr. Seaborg is the author of approximately 200 scientific papers, including a number of comprehensive reviews and compilations in scientific publications. He is also author and co-author of several books on chemistry and the elements.

Honours include: in 1947 named as one of America's 10 outstanding young men by the U. S. Junior Chamber of Commerce; 1947 recipient of the American Chemical Society's Award in Pure Chemistry; 1948 John Ericsson Gold Medal by the American Society of Swedish Engineers; 1948 Nichols Medal of the New York Section of the American Chemical Society; 1953 John Scott Award and Medal of the City of Philadelphia; 1957 Perkin Medal of the American Section of the Society of Chemical Industry; 1959 Atomic Energy Commission's Enrico Fermi Award for his outstanding work in the field of nuclear chemistry and for his leadership in scientific and educational affairs; in 1962, named Swedish American of the Year by Vasa Order of America, Stockholm; 1963 Franklin Medal of the Franklin Institute, Philadelphia.

Dr. Seaborg is an Honorary Fellow of the Chemical Society of London and of the Royal Society of Edinburgh. He is a Fellow of the American Institute of Chemists, the New York Academy of Sciences, the California Academy of Sciences, the American Physical Society and the American Association for the Advancement of Science. He is a Member of the National Academy of Sciences, the American Academy of Arts and Sciences, the Royal Society of Arts of England, and the Royal Swedish Academy of Engineering Sciences.

Honorary degrees awarded to Dr. Seaborg include Doctor of Science degrees from the University of Denver, 1951; Gustavus Adolphus College, 1954; Northwestern University, 1954; University of Notre Dame, 1961; Ohio State University, 1961; Florida State University, 1961; University of Maryland, 1961; Temple University, 1962; Tulane University, 1962; Drexel Institute of Technology, 1962; Georgetown University, 1962; University

of the State of New York, 1962; Mundelein College, 1963; and Trinity College, 1963; the degree of Doctor of Laws from the University of Michigan, 1958; and University of Massachusetts, 1963; the degree of Doctor of Humane Letters from Northern Michigan College, 1962; the degree of Doctor of Public Service from George Washington University, 1962; and the degree of Doctor of Public Administration from the University of Puget Sound, 1963.

In 1942, Dr. Seaborg married Helen L. Griggs, then secretary to the late Dr. Ernest O. Lawrence (Nobel Laureate for Physics 1939). They have six children: Peter (b. 1946), Lynne (b. 1947), David (b. 1949), Stephen (b. 1951), John Eric (b. 1954), and Dianne (b. 1959). His chief hobby is golf, but he also follows other sports with interest. From 1953–1958 he served as the University of California (Berkeley) Faculty Athletic Representative.

Chemistry 1952

ARCHER JOHN PORTER MARTIN

RICHARD LAURENCE MILLINGTON SYNGE

«for their invention of partition chromatography»

Chemistry 1952

Presentation Speech by Professor A. Tiselius, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Your Royal Highnesses, Ladies and Gentlemen.

This year's Nobel Prize in Chemistry is awarded for the discovery of a method for the separation of substances from complicated mixtures.

How can it happen, one may ask, that something apparently so commonplace as a separation method should be rewarded by a Nobel Prize? The answer is that from the very beginnings of chemistry until our own time, methods for separating substances have occupied a key position in this science. Even today, in Holland, chemistry is called « Scheikunde », or « the art of separation », and even today some of chemistry's most important advances are linked to the invention of new methods for separating various substances.

Chemistry today is to a large extent concentrated upon the study of natural products, which are obtained from animals, plants, or even bacteria and other microorganisms. A starting material of this type contains a great number of widely varied substances, some simple, others more complicated. The first thing the chemist must do is to isolate the substances he is interested in from the material and prepare them in a pure state. The next step is, if possible, to identify these substances and find out what they consist of and how they are built up from simple constituents.

The first problem, the isolation, can indeed be difficult, as it is often a matter of preparing in a pure state substances which constitute only an extremely small fraction of the starting material and which have the disagreeable tendency of, so to speak, disappearing between one's fingers when one tries to get hold of them. It is here that Martin and Synge's method has enjoyed great success, especially in what is perhaps its most important form, and is called filter-paper chromatography. A drop of a liquid containing the substance to be investigated is allowed to fall onto a strip of filter paper, where it forms a little spot. This paper is then caused to draw up some suitable mixture of liquids, for example butyl alcohol–water, by capillary action. The spot begins to move, and one can see how it then gradually segregates into several spots, some of which rapidly follow the liquid which has been drawn

up, while others lag behind. Thus there results a resolution of the mixture into its component parts, a resolution which in the last analysis depends on the different *partition* of the substances between the water held by the filter paper and the freely moving butyl alcohol. Hence the name *partition* chromatography. Instead of resorting to a series of intricate chemical operations, one can in this simple way make a complete analysis of even the most extremely complicated mixtures; and a single drop of the starting material is fully sufficient for the purpose.

The method of Martin and Synge, in different forms, has already found extensive application in all branches of chemistry and important discoveries have been made with it. New and interesting substances have been traced and isolated with its help. Metabolic pathways in the organism can be studied and formerly unknown intermediary products identified. This has been done, for example, with studies of the way in which the green leaves of plants build up starch out of the carbon dioxide of the air. With Martin and Synge's method, Calvin and his co-workers in Berkeley have been able to identify some of the most important links in this process, perhaps the most important chemical reaction on our planet.

Partition chromatography has had other extremely important applications when it has been used as a means of studying the structure of giant molecules. It has been possible in this manner to attack problems of the structures of proteins and carbohydrates with considerably greater prospects for success. The intact molecule is too complicated for us to be able to penetrate its structure by chemical methods. If one breaks down these large molecules, a mixture of molecular fragments of different sizes and different chemical natures results. A separation of such extremely complicated mixtures of rather similar substances and an identification of every fragment is a way which allows us to draw conclusions about the structure of the original molecule in the same manner that an archeologist among the ruins of an ancient temple can find archeological details which make it possible for him to some extent to reconstruct the building in its original condition. As a guide in such work, the method of Martin and Synge is of the greatest value. Synge has shown this in some very beautiful investigations on the structure of gramicidin, an antibiotic active against certain bacteria. In work such as this, it is of special importance that the method is suitable for the isolation not only of the smallest building blocks (the amino acids) but also of larger fragments (peptides). It is as if one should have a puzzle to put together: if by chance several pieces happen to hang together, the problem immediately becomes

more simple. The young English chemist Sanger has recently succeeded in putting together an unusually difficult puzzle of this sort; from the mixtures which were separated by Martin and Synge's method, among others, he has been able to get an almost complete picture of the structure of the *insulin* molecule – a result which perhaps more than any other shows the method's great scope and principal significance.

For their part, Martin and his co-workers have recently expanded the method's field of usefulness in other directions, of which experiments with gases and vapours on the one hand and experiments concerning the separation of mixtures of very large molecules on the other are attracting special interest.

Chromatographic analysis has long been known as one of chemistry's most valuable methods. It was discovered in 1906 by the Russian-Polish Michael Tsvett, who succeeded in separating the different pigments in an extract of green leaves using this method. Even earlier than this, several investigators, among them Runge, Schönbein, and Goppelsroeder, had used filter paper for a kind of chromatographic analysis, while Tsvett and many of his successors used mainly tubes packed with various finely powdered, active substances for separations. The novelty in Martin and Synge's method is thus not the «chromatographic column» or «filter paper analysis», but rather concerns the fundamental chromatographic process itself. This can now be formulated as the partition of a substance between two liquids, instead of – as previously – entirely as its concentration at the surface of a more or less poorly defined active powder. Thus we have a rational basis for the method and enormously larger possibilities for choosing the experimental conditions which will be most suitable in any particular case. The almost explosive development of chromatography since the discovery of Martin and Synge's principles shows the power and scope of their invention.

Dr. Archer Martin, Dr. Richard Synge. Your invention of partition chromatography has given to science a new tool which already has proved its usefulness in an impressive number of important investigations.

This tool has enabled research workers in chemistry, biology and medicine to tackle and solve problems which earlier were considered almost hopelessly complicated.

However, Nature *is* complicated, and this is particularly characteristic of substances and reactions which determine Life. The structure and the functions of the complex giant molecules of the living cell are in the focus

of interest of to-day's research. It has been a great experience to all involved in such research to witness how much the study of these very complex problems has benefited from the very simple tool invented by you. I believe it is in the best British tradition to make great discoveries with simple means. Our high appreciation of your achievements is further strengthened by the fact that this pioneer work of yours was done during the first years of the World War, thus at a very difficult time for your country and your people.

It is a great satisfaction to The Royal Swedish Academy of Sciences to award to you jointly this year's Nobel Prize for Chemistry for your invention of partition chromatography. May I offer you our congratulations.

Archer John Porter Martin, Richard Laurence Millington Synge. May I now request you to receive from the hands of His Majesty the King the Nobel Prize for Chemistry for the year 1952.

Archer J. P. Martin

The development of partition chromatography

Nobel Lecture, December 12, 1952

The subject of partition chromatography has now an extensive literature and I feel on an occasion such as this it is better to describe the development of the method rather than to summarize the present position or to attempt to put forward any of the more recent advances, which have been described elsewhere. Perhaps indeed, if enough histories, written while the ideas are still fresh in the minds of the people concerned, are available for a variety of discoveries or inventions, it may eventually be possible to lay down some of the principles required to facilitate the obtaining of fruitful results in scientific research in general. Clearly also the background of knowledge at the time the advance was made will be best understood if the history is as recent as possible.

Partition chromatography resulted from the marrying of two techniques, that of chromatography and that of countercurrent solvent extraction. All of the ideas are simple and had peoples' minds been directed that way the method would have flourished perhaps a century earlier. In fact the minds of laboratory workers seem to have been closed to countercurrent procedures, which were adopted in industry, e.g. in distillation and lixiviation, long before they were generally used in the laboratory. In industry the use of the countercurrent principle led to great economies in heat and solvents, and its value was obvious: in the laboratory such economies are of comparatively little importance and the more subtle point that by its aid a vast improvement in certain difficult separations could be effected was not appreciated. Indeed it is only within the last twenty years that high efficiency distillation apparatus has been available in the laboratory and twenty-five years elapsed after Tsvett's classical work on chromatography before the latter method was put to general use. Synge and I were fortunate to work at a time when the need for new methods was apparent to many people, so that our methods have gained general acceptance in ten years.

I was fascinated by fractional distillation as a method while still a schoolboy, and built in the cellar of my home, which was my combined workshop and laboratory, distillation columns, packed with coke of graded size, some

five feet in height. They were made from coffee tins (obtained from the kitchen), with the bottoms removed, soldered together! Experience with them served me in good stead and by the time I graduated I had a good understanding of the problems of fractional distillation.

When later I began to do research at the Dunn Nutritional Laboratory on the attempted isolation of vitamin E, I applied similar ideas to the problem of extracting into ether the unsaponifiable matter from saponified wheat germ oil. The apparatus consisted of a twenty litre aspirator bottle placed on the flat roof of the building, connected at its bottom tubulure by means of a half inch glass tube with a twenty litre bottle. The bottom vessel was filled with ether, the top with the soap solution. This was set up in the afternoon and by the next morning the liquids had interchanged their position. Experiment showed that there was an extraction efficiency corresponding to about ten theoretical plates.

The next stage followed the reading a single sentence at the end of an American paper on the purification of Vitamin E, that a countercurrent fractional extraction machine was giving an improved product. Until then it had not occurred to me that an extraction column could be used to separate two substances of rather similar partition coefficient. I can still vividly remember the satisfaction of working out how this could be done using a long column with the material to be analysed injected in the middle, the two halves of the column, as it were, opposing each other, the ratio of rates of flow of the two solvents being chosen to lie between the reciprocals of the partition coefficients of the substances to be separated, so that the two substances left the column, one at one end and the other at the other end. I worked out the theory of the columns, unaware because of my deficient reading, that it had mostly been done before. I recognized that it could be done in a succession of separating funnels, but the labour involved was prohibitive for any large number.

It was then I think I discovered what I might call Martin's principle of scientific research, viz. « Nothing is too much trouble if somebody else does it ». Now this is a very difficult principle to apply, particularly if one is a Ph.D. student. Since, however, it is immaterial whether the work is done by assistants or a machine, I decided to build a machine equivalent to an array of about 200 separating funnels and to use essentially the same arrangement as I had used in the extraction of Vitamin E from the soaps. This required in essence a tube some 60 metres long, and I devised a method of breaking up the tube into a number (45) of vertical sections each united to its neighbours

by two pairs of small-bore glass tubes, each containing a ball valve. Fig. 1 shows a diagram of a single unit.

A diaphragm pump with adjustable valves pumped into the column the two phases, one at either end and also provided an alternating flow which circulated the liquid from the bottom of one main tube to the top of the next through the small-bore tubes. Fig. 2 shows a picture of this machine and the author at the time. Using methanol and 60°–80° boiling point petroleum ether this machine had an efficiency of about two hundred theoretical plates. It was, however, like the American machine due to Cornish (details of which were published before I had finished mine), before its time. I could not obtain support to continue work on it and after using it for a Vitamin E purification, in the course of which I obtained the first evidence of the multiple nature of Vitamin E, the machine was never used again. Since I failed also to publish my Vitamin E results the whole of the effort put in this work appeared to have been wasted.

The next two years I spent looking after pigs on diets deficient in the B_2

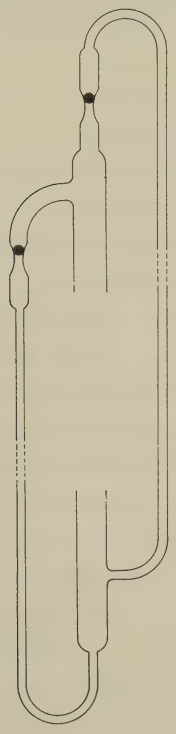


Fig. 1.

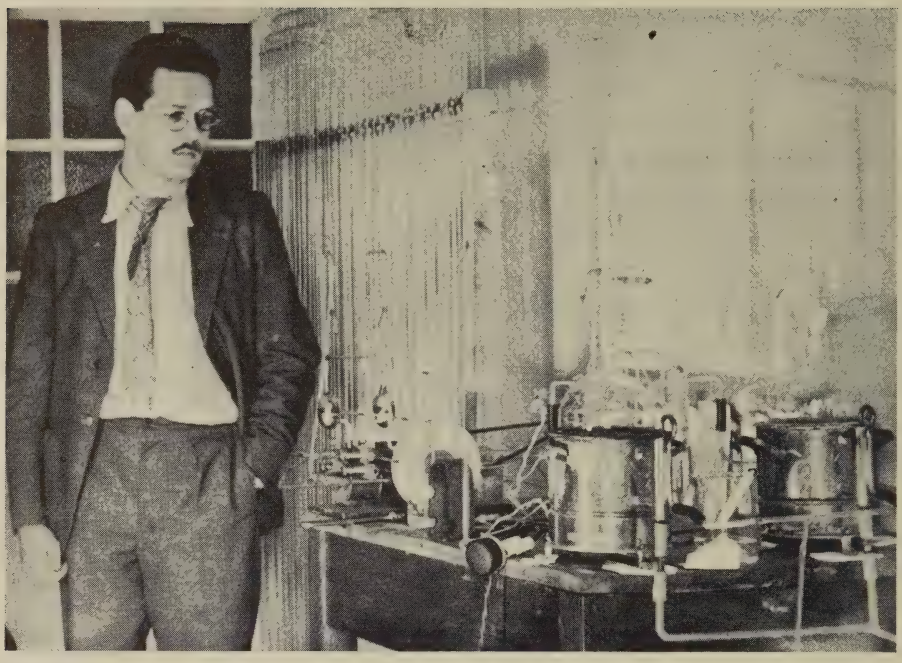


Fig. 2.

group of vitamins. This work was done under the close supervision of Sir Charles Martin, whose guidance has been of inestimable value to me. It was he who suggested that I should meet Synge and perhaps collaborate on the problem of separating acetyl amino acids.

Synge had already shown that the acetyl amino acids had partition coefficients between chloroform and water, which were different enough to permit of good separations if a high efficiency countercurrent extraction machine were available. Unfortunately the machine I had built was not suitable for chloroform and water, and methanol and petrol ether were not very good for the acetyl amino acid separation.

We decided, therefore, to build another apparatus for the purpose. I built a rough three plate machine of entirely different type, with stirring and settling compartments, as a model for a forty plate machine, which we decided would suffice for our needs. Synge had the forty plate model constructed in the Biochemical Laboratory at Cambridge. Meanwhile I had moved to the Wool Industries Research Laboratories at Leeds. The machine when complete needed modification, so Synge moved to Leeds so that we

could work on it together. After a lot of work we were able to determine the more fatty amino acids in wool, with much greater accuracy than had been done before. The machine, however, was very troublesome to use and required nursing day and night for several days at a time.

A better machine was needed before the method could be of general application, and I made dozens of new designs, which I put up in turn to Synge, who became very tired of listening to me. But none of them seemed easy to make, and no construction was undertaken.

I then had an idea of a radically different kind. This was to pack a tube with a mixture of wool and cotton, with the fibres parallel to the axis of the tube. Now cotton is wetted preferentially by water and wool by chloroform. By putting chloroform above and water below I hoped that I should obtain a large number of parallel streams of the two solvents with a large area of contact. When I tried it out I was very disappointed to find that large domains went over to one solvent or the other and the arrangement was actually less efficient than an unpacked tube.

Up to this time my thinking had been dominated by the idea of moving the two phases in opposite directions simultaneously. But in considering the failure of the cotton and wool experiment I realized that it should be comparatively simple to hold one phase stationary and move only one. The arrangement would then be essentially a chromatogram. I put this idea to Synge and he was full of enthusiasm, and we decided to work on it at once.

We decided to try first silica gel, of the type used to dry balance cases, suitably ground up, to hold the water, and having packed a chromatogram tube with this, to run chloroform down the column. We tried first the separation of acetyl alanine and acetyl leucine. We were then faced with the task of determining what was happening and how to decide whether separation had occurred or not. We quailed at the thought of evaporating down and titrating a large number of cuts and the idea occurred to us of including in the water in the silica gel an indicator with an appropriate range to show the presence of the acetyl amino acid. We chose methyl orange and the first column we made up showed the separation we looked for, as bright red bands on an orange column.

We were greatly encouraged by this and resolved to do the experiment with more care. We carefully purified our chloroform. Then the red bands stuck at the top of the column, as a result we presumed of adsorption on the silica gel; indeed addition of alcohol to the chloroform made the column

behave as before. Alcohol was, of course, present as a stabilizer in the commercial chloroform we used in the first experiment.

I would like to take this opportunity to record here my belief that one should take a minimum of care and preparation over first experiments. If they are unsuccessful one is not then discouraged since many possible reasons for failure can be thought of, and improvements can be made. Much can often be learned by the repetition under different conditions, even if the desired result is not obtained. If every conceivable precaution is taken at first, one is often too discouraged to proceed at all.

We soon found that a batch of commercial precipitated silica gave better columns, particularly after some fatty material had been extracted with alcohol. When, however, this batch came to an end we were unable to make the new batch work at all. The colour of the methyl orange was too red to show any change in the presence of the acids, and if the colour was made more yellow by addition of a base, though well-defined bands were seen at first, their intensity decreased as they moved down the column, and a large part of the acetyl amino acid was found to remain on the column. We then attempted to prepare our own precipitated silica by as many methods as we could think of, and finally arrived at an empirical method for its preparation from sodium silicate, which was reasonably satisfactory. It is of interest to note that in this method we found it necessary to carry out the precipitation in the presence of methyl orange. Dickey has since shown that an increased affinity for methyl orange can result from this; which was perhaps of assistance to us in preventing the methyl orange from being leached from the column by certain solvents. We found it was necessary to « age » the silica by standing with dilute hydrochloric acid for a day or two, in order to obtain a colour of column on which the acid zones were observable, but this ageing process could not be continued too long without so increasing the adsorptive power, that separation of the fast moving acids was unsatisfactory.

This method however has not been found to be satisfactory with other batches of sodium silicate, and an empirical approach has still to be made to the preparation of precipitated silica for any particular use.

It is of interest to consider how the method would have developed had we not thought of using an indicator on the column. The indicator enabled us to work rapidly and do many experiments, which were needed in the early stages, but most of our difficulties were due to the need to obtain a silica which gave a suitable colour with the indicator, and at the same time satisfactory separation of the acetyl amino acids. Had we thought of the titration

techniques such as are used at present, where hundreds of titrations can be carried out without undue labour, the problems to be solved would have been simpler, and the more extensive information gained of the behaviour of the column would have greatly improved the final method. I think it is indeed possible that the acetylated amino-acid method might have had a permanent place in analysis had we started with this technique. But at the time it seemed an unnecessary waste of labour to titrate hundreds of cuts from a single column when we thought we could collect each band separately.

About the same time at least two other investigators conceived essentially the same idea as we had had for this « partition chromatography ». I refer to Sverre Stene in Norway and Van Dyck in Holland. They had, however, no problems they were immediately able to solve with its aid and consequently their work has passed comparatively unnoticed.

Since we had approached the partition chromatogram from the direction of fractional extraction, it was natural to apply an analogous type of theory to its operation. Once the concept of the height equivalent to a theoretical plate (H.E.T.P.) had been clarified (somewhat similar to that introduced by Peters for distillation in packed columns) it was easy to give the theory of the chromatogram for the case where the partition coefficient remained constant, and to guess qualitatively the result where the partition coefficient, because of ionisation, association or absorption on the silica, depended on the concentration.

Table 1.

	R	α *Direct*	α *Column*
Acetyl proline	0.37	9.5	9.4
Acetyl phenyl alanine	1.07	1.3	1.4

(Solvent $CHCl_3$ + 1% butanol.)

Synge and I were able to show that to a first approximation, the mono amino mono carboxylic acids behaved, under proper conditions in accordance with theory. They were not appreciably adsorbed by the silica, and the water in the silica had approximately the solvent powers of ordinary water. Table 1 shows the comparison of the partition coefficients deduced from the behaviour on the column and those measured directly for acetyl proline and acetyl phenyl alanine.

Gordon, Synge and I developed the method for the analysis of the seven more fatty mono amino mono carboxylic acids using two types of column (Table 2).

Tristram continued with further work on this and his recoveries are shown in Table 3.

Gordon, Synge and I were then unable to extend the method to other amino acids, and turned our attention to the separation of the amino acids them-

Table 2.

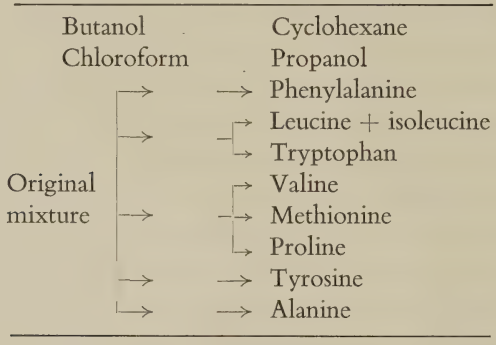


Table 3. Percentage recovery of acetylamino acids from mixture of amino acids resembling hydrolysate of insulin (Tristram).

	24-hours hydrolysis	
	before	*after*
Phenylalanine	99 ± 2	96 ± 2
Leucine + isoleucine	102 ± 2	94 ± 2
Valine	98 ± 2	98 ± 2
Proline	93 ± 3	93 ± 3
Alanine	102 ± 2	104 ± 2
Tyrosine	95 ± 2	95 ± 2

selves, and not their acetyl derivatives. Here indicators could not be used on the column and a method of running a sewing thread beneath the column, (so that it was wetted by the effluent) and then winding it on to a slowly rotating screwed rod was tried. On treating the thread on the screwed rod with ninhydrin a record of the behaviour of the column was obtained. Using butanol and water, as suggested by the work of Dakin, and England and Cohn, we found that great absorption of the amino acid by the silica in-

terfered with the separations. The adsorption was of the Freundlich type causing a long tail to the bands.

Since, however, we could no longer use an indicator we were able to try again those substances as supports for water, which we had previously abandoned because we were unable to obtain a suitable change of colour of the indicator. We were thus led again to try cellulose as the water-holding medium.

I was already familiar with the use of filterpaper chromatograms as used by the dyestuff chemists and adopted at first their technique. A ten centimetre circular paper was cut to a semicircle with a three centimetre tail about one centimetre wide at the centre. On this tail near the centre was placed a drop of a solution of valine and leucine. The tail was put in butanol saturated with water contained in a Petri dish surrounded by water saturated with butanol in a glass jar. The semicircle of paper rested on the edge of the Petri dish. The top of the jar was covered with a glass plate. When the butanol had reached the edge of the paper it was taken out and dried and dipped in a solution of ninhydrin in ether. On heating two semicircles of colour were observable, and it was evident the method was a success. It was found quicker and more convenient to hang strips of paper from troughs containing the solvents, and this method was adopted as a routine. There followed a period in which we tried all the solvents we could lay our hands on, and logged the positions of the various amino acids in each of these. Experiment showed that the theory we had put forward for the silica columns applied equally well to paper chromatograms. It was indeed surprising with what closeness the solvent power of the water in the paper followed that of free water.

No one solvent, however, was able to resolve a mixture of all the common amino acids, and I tried to separate into acidic, neutral and basic fractions by ionophoresis, prior to separation in a direction at right angles by chromatography. The method adopted was rather clumsy. Each end of a square paper was treated with paraffin wax so that a centimetre wide strip of untreated paper was left, nearer one end than the other. This strip was moistened with acetate buffer and a drop of amino-acid mixture put in the middle. Copper wires were applied to either end of the strip and a potential of two hundred volts applied. After a couple of hours the paper was dried, the paraffin wax removed with petroleum ether and the paper developed as a chromatogram with water saturated butanol. A two dimensional separation of the amino acids was revealed on spraying with ninhydrin and heating. The

separation however of the neutral group of amino acids was not satisfactory. Attention was therefore concentrated on chromatography successively in two directions at right angles.

This two dimensional development of paper chromatograms, of the classical not the partition type, had already been done by Liesegang, but I was unaware of this until just lately.

At this stage Synge left us to go to the Lister Institute and I continued work with Consden and Gordon. At first finding good systems for two dimensional chromatograms was our main preoccupation. One problem caused us much worry. With butanol, phenol or benzyl alcohol, but not with isobutyric acid or collidine, the amino acids, particularly the faster moving ones, disappeared as they moved down the paper. As well as a purple spot, a continuous streak, starting from it but moving faster, of pale pink became visible after ninhydrin treatment. Sometimes the purple spot disappeared altogether. We thought this must be the result of oxidation or some other decomposition. Excluding oxygen did not improve matters. Then we found that phenol with ammonia was a particularly useful solvent, but here the paper rapidly blackened. We found in the literature that this was due to catalytic oxidation in the presence of copper. It was readily possible by including in the solvent or the atmosphere substances which formed complexes with copper, to eliminate this blackening completely. Simultaneously the « pink front » phenomenon disappeared. Thereafter we used a beaker of potassium cyanide in the bottom of the chromatogram box, or used a box which could be completely filled with coal gas.

There is enough copper in most papers to be able to show the effect, but in our case a large amount of copper was present in the atmosphere of the laboratory as we were using a motor in the drying cabinet, whose commutator sparked badly. (The copper complexes of the α-amino-acids do not give compact spots on paper and separation of the copper complexes is much more difficult than the separation of α-amino-acids. Dent, however, has shown that this complex formation on paper affords a ready method of distinguishing α from other amino acids.)

The high sensitivity of the ninhydrin reaction made it possible to do a chromatographic analysis with a few micrograms of any amino acid. So little labour was involved that it was possible for hundreds of analyses to be done simultaneously. Even in the case of unknown substances, something could be inferred of their nature from their behaviour in different solvents.

We were convinced that the method was by no means confined to amino

acids. Partridge spent a day or two in our laboratory and a few days after his return to Cambridge, wrote to us and told us that he had been able to separate reducing sugars, and detect them with silver nitrate. He found that practically the same solvent systems were satisfactory for sugars and amino acids. Later he worked out the methods for carbohydrates as completely as we had done for amino acids.

We found also that the method worked well for the simpler peptides, and decided to attempt to learn something of the structure of keratin, by the examination of partial hydrolysates of wool. This involved developing techniques for preliminary group separation of the peptides before the application of chromatography, since the mixture to be separated was of such extreme complexity. We developed the method of ionophoresis in silica jelly for this, preferring it to a chromatographic method because the fractions obtained would not then be chromatographically similar. We had also to work out the technique of washing peptide spots from the chromatogram, hydrolysing and returning to another chromatogram. We were able to do this without very serious losses using capillary tubes as vessels and a sheet of polythene, on which a drop of liquid stays, compact for evaporation and solution. We were able also to de-aminate the terminal NH_2 group of a number of simple peptides to permit determination of nature of the end amino acid.

Synge at this time was working on the structure of gramicidin-S which he showed to be a cyclic polypeptide with one each of five different amino acids. This was an excellent test material for these methods; in collaboration we were in fact able by them to determine the order of the acids. Confirmation of this order was obtained using Sanger's dinitrophenyl end group method.

Sanger, in his recent remarkable achievement of the complete determination of the order of linking the amino acids in insulin, has shown how these methods may be developed for the much more difficult problem of the structure of a protein.

Elsden and Synge showed that potato starch could be used to pack a column and gave essentially the same separations of amino acids as did paper sheets. Moore and Stein showed that with careful control a complete quantitative analysis of a protein could be performed on starch columns using a ninhydrin colorimetric method. They also introduced the use of automatic fraction collectors which are now so widely used. Later the starch columns were superceded by ion-exchange resins. Their methods now provide the best means of protein analysis.

Many independent attempts have been made to put paper chromatography on a quantitative footing. Various sources of error have been discovered but so far the accuracy is inferior to the Moore and Stein method, though it may suffice for many purposes.

I will now return to the silica columns. For the acetylamino acids we used water as the stationary phase. So also did Elsden, who adapted it for the lower fatty acids. Isherwood used sulphuric acid instead for the separation of hydroxy and dicarboxylic acids, since it was desirable to suppress their ionization. Levi used a buffer for penicillin separations, in this case to obtain a given proportion of the penicillin in the ionized form. These penicillin columns were widely used and kieselguhr rather than precipitated silica was found satisfactory in America. Its great advantage was that it was available commercially and was less variable than precipitated silica, and less likely to cause trouble due to adsorption. To get good columns, however, a new technique of packing the columns was required. This technique is not, however, difficult and I consider that for most purposes precipitated silica is now obsolete. Kieselguhr has an advantage in another respect. The pore size of precipitated silica is small enough to preclude its use for very large molecules, whereas that of the kieselguhr is relatively almost infinite. In fact, Porter and I have shown that it is possible to separate ribonuclease into two different fractions on a partition chromatogram using kieselguhr as support. Admittedly this is a small and atypical protein but Porter has extended its use to a few other proteins.

The surface of silica is wetted preferentially by the more polar of a given pair of liquid phases in equilibrium. So that in the usual column the mobile phase has to be the less polar one, and only those substances whose partition coefficient is in favour of the less polar phase, can be satisfactorily separated. By treatment of the silica with a silicone or silane the surface becomes preferentially wetted by the less polar phase. A « reversed phase » column thus becomes possible, suitable for the separation of substances which favour the less polar phase.

The first « reversed phase » chromatogram was a paper one loaded with rubber latex, and was due to Boldingh. He also used columns packed with powdered rubber, for the separation of the higher fatty acids. Paper alone or silane treated, will hold a liquid hydrocarbon and give satisfactory chromatograms, with say aqueous alcohols as the moving phase.

Finally, I would like to mention gas–liquid chromatography. Synge and I suggested the possibility of this ten years ago in our first paper on the parti-

tion chromatogram. No one seems to have taken it up until James and I did so recently, to try and encourage ourselves after a very unsuccessful attempt to put fractional crystallization on a column basis. These chromatograms differ from the usual ones only in that the mobile phase is a gas instead of the usual liquid. They are suitable of course only for volatile substances.

Because gas is so mobile compared to liquid it is feasible to use much longer and thinner columns, and since diffusion is so much more rapid, to move the gas rapidly without increasing the H.E.T.P. It is thus possible to use columns with a very high separating efficiency. So far our work has been confined to substances which can be titrated, acids and amines. A more general method of detecting vapours in the gas issuing from the chromatograms should, however, make this type of column available for use with all types of substances that can be distilled at a pressure of a few millimetres of mercury. Whereas, with distillation, apparatus has at most several hundred theoretical plates, gas chromatographic columns with one to ten thousand plates are readily possible, and the quantity of material required for analysis is milligrammes instead of grammes.

It is very easy with the chromatographic apparatus to measure the free energy of solution of a given volatile substance in the stationary phase. I hope it will be possible to learn with its aid a great deal about the molecular forces involved in solution.

Biography

Archer John Porter Martin was born on March 1st, 1910, in London where his father was a general medical practitioner. He attended Bedford School from 1921 to 1929 when he entered Cambridge University to graduate in 1932. After a year in the Physical Chemistry Laboratory he obtained a post at the Dunn Nutritional Laboratory, where he worked under L. J. Harris and Sir Charles Martin, and in 1938 he moved to the Wool Industries Research Association at Leeds. From 1946 to 1948 he was Head of the Biochemistry Division of the Research Department of Boots Pure Drug Company at Nottingham and in 1948 he joined the staff of the Medical Research Council, first at the Lister Institute and later at the National Institute for Medical Research. He was appointed Head of the Division of Physical Chemistry at the Institute in 1952 and he was Chemical Consultant from 1956 to 1959. Since 1959 he has been a Director of Abbotsbury Laboratories Ltd.

Martin entered Cambridge University with the intention of becoming a chemical engineer but, due to the influence of Professor J. B. S. Haldane, then Reader of Biochemistry at Cambridge, he eventually specialized in biochemistry. His first researches, as an undergraduate, resulted in a method of detecting pyro-electricity by observing the attraction of a metal plate for crystals that had been immersed in liquid air. At Cambridge he worked on ultraviolet adsorption spectra and at the Nutritional Laboratory he was concerned with the attempted isolation of Vitamin E and in the pathological effects of prolonged Vitamin E deficiencies. In these latter studies he used solvent extraction and chromatographic methods which were to lay the foundation for his later work on chromatography. He also worked, along with others, on the B_2 group of vitamin deficiencies in pigs.

At the Wool Industries Research Association he worked on the felting of wool, first with R. L. M. Synge and later with Consden and Gordon, and on amino-acid analysis. It was here that he developed his method of partition chromatography; more recently, with A. T. James, he has developed the method of gas-liquid chromatography.

Dr. Martin, a Fellow of the Royal Society (1950), was made Companion

of the British Empire in 1960. He received the Berzelius Medal of the Swedish Medical Society (1951), the John Scott Award (1958), the John Price Wetherill Medal (1959), the Franklin Institute Medal (1959), and the Leverhulme Medal (1963).

In 1963, he was appointed to deliver special lectures (as « buitengewoon hoogleraar ») at the Technological University of Eindhoven, The Netherlands.

In 1943 he married Judith Bagenal; they have one son and three daughters.

Richard L. M. Synge

Applications of partition chromatography

Nobel Lecture, December 12, 1952

This lecture is intended to depict some of the applications and consequences of the invention of partition chromatography. Before embarking on this, though, I should explain how I came to be taking part in the collaborative work which Dr. Martin has just described.

While still at school I heard tell of the science of biochemistry, which should draw together two aspects of nature into a common study. I had long been fascinated by living things (particularly the wild plants), and more recently by chemistry. This last was probably more for the appeal to the senses of the chemist's activities than for the knowledge of the nature of things which chemistry imparts. It was particularly reading in the newspaper Sir Frederick Hopkins' presidential address to the British Association for the Advancement of Science at Leicester in 1933 that impressed on me the idea that living things must have wonderfully precise and complicated working parts on the molecular scale and that the biochemist had the best chance of finding out how these are put together and do their work. As I was to begin studying natural science at Cambridge University in the same year, the ambition of studying in Prof. Hopkins' laboratory could easily be realized. Nevertheless, experiences in that laboratory in the elementary course taken by medical students did not give the impression of biochemistry being a refined or precise science. It was with a rather blind faith in the ultimate justification of Hopkins' ideas that, after several months of indecision, I embarked on the advanced course in his laboratory (Part II of the Natural Sciences Tripos). Here, immediately, one came in contact with exciting facts and exciting ideas. The latter outnumbered the former, usually. However, at an early stage in the course the student engaged in some quite rigorous isolative work under the guidance of Mr. N. W. Pirie. Pirie used to enliven the long hours at the bench with caustic anecdotes from the history of biochemistry which helped, quite as much as the isolative work itself and his comments thereon, to develop the critical faculty in those who possessed a rudiment of it. On graduating in 1936 I continued in the laboratory as a research student under

Pirie's supervision, and he suggested that I should make a chemical study of the glycoproteins, a class of substances then, as now, obscure in chemical nature and of great physiological interest. Soon I found my knowledge both of carbohydrate and protein chemistry was inadequate to the task, and began some synthetic work with Dr. D. J. Bell which involved preparing partially substituted derivatives of glucose. Among many useful things, I learnt from Bell the power of liquid–liquid extraction, with and without salting out, for separating methylated sugars according to the extent of their methylation. I returned to work on glycoproteins, with the idea of studying them by methylation. This brought me into contact with Dr. A. Neuberger, who was then working in Prof. C. R. Harington's laboratory at University College Hospital, London. Neuberger had developed an ingenious method for isolating the carbohydrate moiety from digested egg albumin by *N*-acetylating the digests and then removing *N*-acetyl amino-acids and peptides by exhaustive extraction with chloroform. He then *O*-acetylated the residue, whereupon the acetylated carbohydrates became extractable.

About this time, in 1937, Dr. Hedley R. Marston (then, as now, director of the C. S. I. R. Nutrition Laboratory at Adelaide, South Australia) came to the Biochemical Laboratory in Cambridge for a year as a guest. He was given bench space in the room where Pirie and I were working. He brought with him apparatus much more complicated than most workers in that laboratory were accustomed to, including an «artificial rumen» for microbial digestion of cellulose and a long fractionating column for distilling the esters of the resulting fatty acids. This by no means occupied his whole time, and we were impressed by the large number of letters which he wrote and by the frequent recurrence of his birthdays, which he celebrated by inviting numerous colleagues to very lively scientific discussions in a nearby public house. After about six months I began better to understand Marston's extramural activities when he told me that he was advising an organization called the International Wool Secretariat. This was given funds by the wool growers of Australia, New Zealand and South Africa for the purposes of publicity and research; they were worried both by the contemporary slump and by the advent of various synthetic fibres which it was then thought might prove to be effective substitutes for wool. Marston's advice was to apply some of their money to fundamental studies of the nature of wool, and he suggested that part should be given to me as a Studentship to study in detail the amino-acid composition of wool, beginning by improving the methods of amino-acid analysis. «If you work steadily at that for five years, you will revolu-

tionize the whole of protein chemistry» he said. The Studentship was on unusually generous financial terms, and as I also thought it would fit in with acquiring a more detailed knowledge of the protein side of the glycoprotein problem, I readily agreed. I began work in 1938 by studying the distribution of acetyl amino-acids between chloroform–water phases as a possible analytical procedure, directly suggested by Neuberger's experiments. The partition coefficients showed very encouraging differences, and I was advised from all sides to consult Dr. A. J. P. Martin as to how best to conduct the separations, since his bizarre-looking apparatus for purifying vitamin E at the Dunn Nutritional Laboratory had attracted widespread notice in Cambridge.

Dr. Martin has told you how our collaboration then developed. It might well have been effective in any case, but it was made enormously easier because Martin's appointment at Leeds had also been financed by the International Wool Secretariat and their scientific adviser, Sir Charles J. Martin, kept in close touch at all times with our work. Both during and after the time I was working in Cambridge I received much very valuable advice from Sir Charles and he made it easy for my Studentship to be transferred when it became desirable to join Archer Martin in the laboratories of the Wool Industries Research Association at Leeds.

I will discuss in most detail the application of partition chromatography to the study of proteins. Partly because of intense interest of biochemists in proteins and partly because the earliest work with the method was in this field, the results obtained with partition chromatography have been especially striking here, although the method has similar capabilities in other branches of biochemistry.

First, the method has given the possibility of exhaustive analysis of complete hydrolysates of proteins. For qualitative work the two-dimensional procedures using paper have been most convenient. For quantitative work the procedures using starch grains elaborated by S. Moore and W. H. Stein have permitted accurate and complete analyses on a few mg of hydrolysed protein (Fig. 1). Latterly these workers have found ion-exchange resins to have advantages over starch. Apart from the positive value to protein chemistry of having convenient methods for carrying out the fundamental operations, the use of partition chromatography has helped firmly to establish that the vast majority of proteins yield on hydrolysis only the well-known amino acids and that these account for the entire substance of the protein molecule. This generalization is fundamental for the future devel-

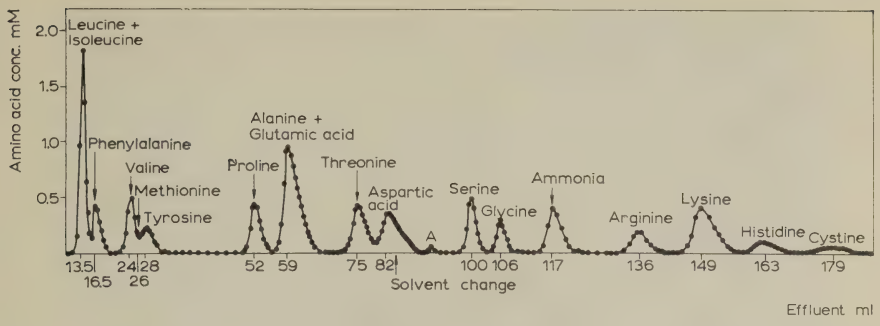


Fig. 1. Separation of a mixture of amino-acids on a starch chromatogram, as shown by analysis of the effluent. (S. Moore and W. H. Stein, *J. Biol. Chem.*, 178 (1949) 53.)

opment of the study of proteins and was too often taken on trust in the past.

In parallel with studies of amino acids in protein hydrolysates, partition chromatography has thrown much light on the free amino acids and related compounds found in living organisms and products derived therefrom. This work has shown how certain compounds are found in the tissue juices of all living organisms. Fig. 2 shows a two-dimensional chromatogram of potato juice, by C. E. Dent and colleagues. A number of new metabolites have been recognized and isolated by these means, and considerable light has been thrown on metabolic relationships, distribution of amino acids in different tissues and so forth. Unfortunately, the standards of identification used by many workers with paper chromatography have often been inadequate and the new meaning of the term « paper chemistry » is often not so far from the old one.

Partition chromatography has likewise been valuable for assessing the purity of amino acids and simple peptides and for studying the actions of enzymes so far as such simpler molecules are concerned. Previously unsuspected « transpeptidations » and syntheses have been revealed by this means.

The method has proved useful for the various procedures for allocating free functional groups within molecules of proteins or peptides; thus at present we have procedures for recognition of free amino groups by substitution with dinitrophenyl and other radicals, and of carboxyl groups by reduction to the corresponding alcohols or by enzymic hydrolysis of terminal amino-acids. These methods nearly always use partition chromatography as a final stage in the identification.

Finally, partition chromatography is of great importance for ascertaining the sequence of amino-acid residues in the peptide chains of proteins. Martin

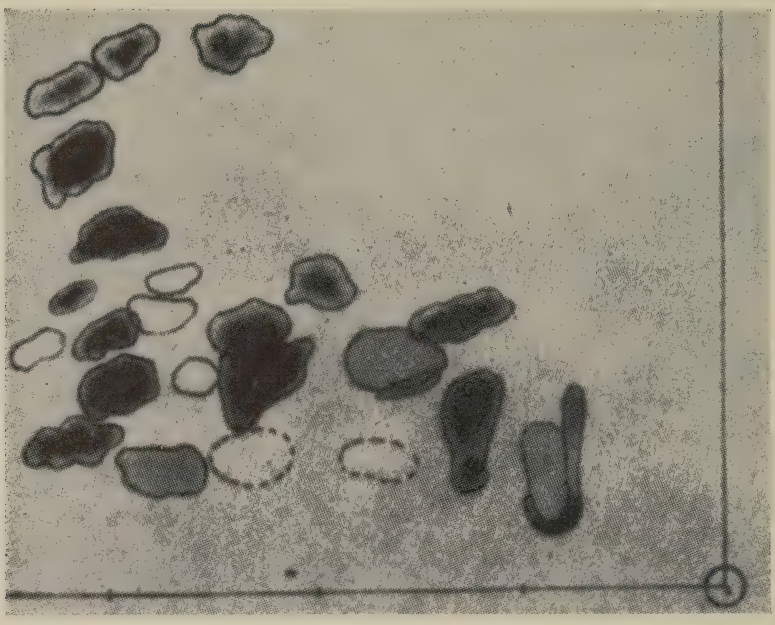


Fig. 2. Two-dimensional paper chromatogram of an extract of potato, coloured with ninhydrin showing free amino-acids, etc. (C. E. Dent, W. Stepka, and F. C. Steward, *Nature*, 160 (1947) 682.)

and I had this use particularly in mind throughout our work. If a peptide chain is partially degraded to dipeptide and tripeptide fragments, etc. it should be possible, by identifying these, to recognize the original compound from which they are derived; thus in a simple case the peptide chain represented A—B—C—D—E will give rise to the fragments A—B, B—C, C—D, and D—E, permitting an unequivocal reconstruction. Martin and I, with R. Consden and A. H. Gordon, were able in this way, mainly using partition-chromatographic methods, to determine the amino-acid sequence in gramicidin-S, which is probably a cyclic decapeptide (Fig. 3). Subsequently F. Sanger and colleagues have elucidated by similar methods what may be the entire peptide sequences in the structure of ox insulin, the minimum molecule of which embodies 51 amino-acid residues. One of the two sequences established is shown in Fig. 4.

In the study of carbohydrates partition chromatography has played a very similar rôle. It can be used for determining ultimate hydrolysis products of polysaccharides qualitatively and quantitatively, it permits analysis of naturally occurring free sugars and their esters, and also the determination of the

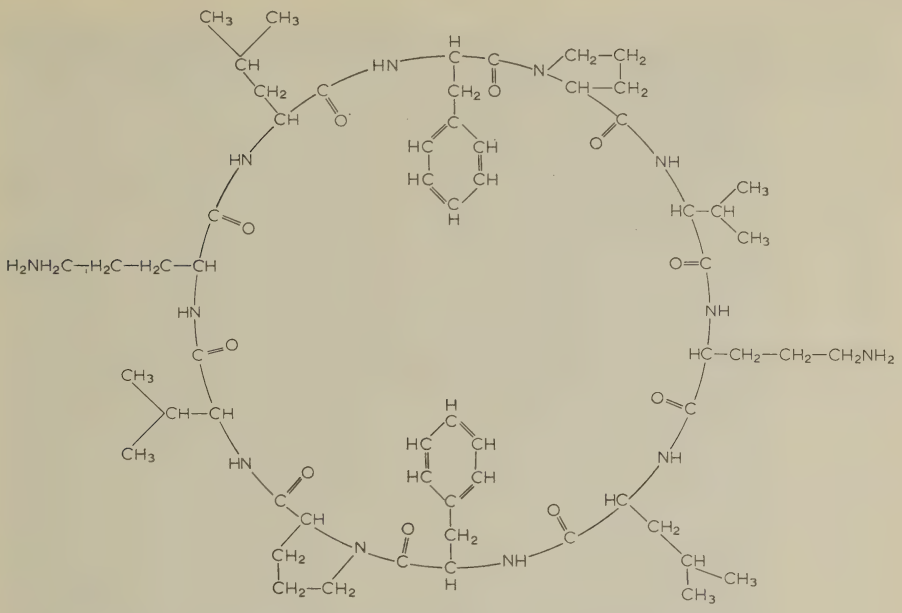


Fig. 3. Probable formula of gramicidin-S.

mode of linkage of the component parts of more complicated molecules by substitution methods or by partial hydrolysis methods. Special mention should be made of the discovery of series of complicated fructosan-like oligosaccharides in the juices of various plants by means of paper partition chromatography (cf. Fig. 5). Partition chromatography has also proved almost ideally adapted for analysing the hydrolysis products of methylated polysaccharides, and permits more accurate analysis with much smaller quantities of material, thereby greatly increasing the scope of the methylation method as a step in determining the structure of natural polysaccharides. It gives me great pleasure that my teacher D. J. Bell was the first to use the method for this purpose.

It is evident that partition chromatography has considerable application in the study of the nucleic acids and their breakdown products, the third great chemical family of biological importance. Here the method can fulfil a similar group of functions. However, owing to the ionic nature of so many of the compounds concerned, methods using electrical transport or chromatography on ion-exchange materials seem likely to assume greater importance with these compounds.

With the lipides there is not the problem of chemical study of giant mole-

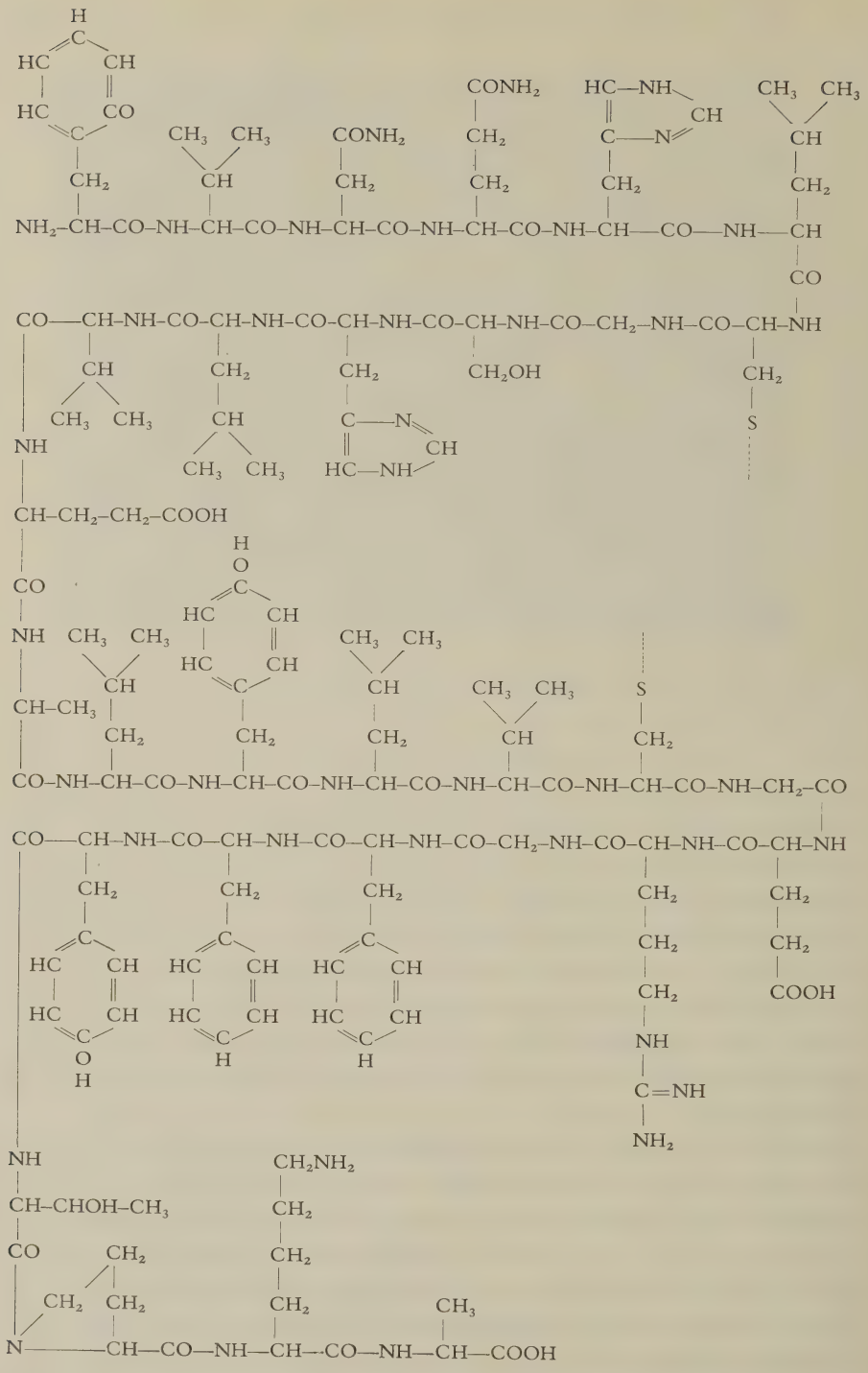


Fig. 4. Probable formula of the phenylalanyl chain in ox insulin.

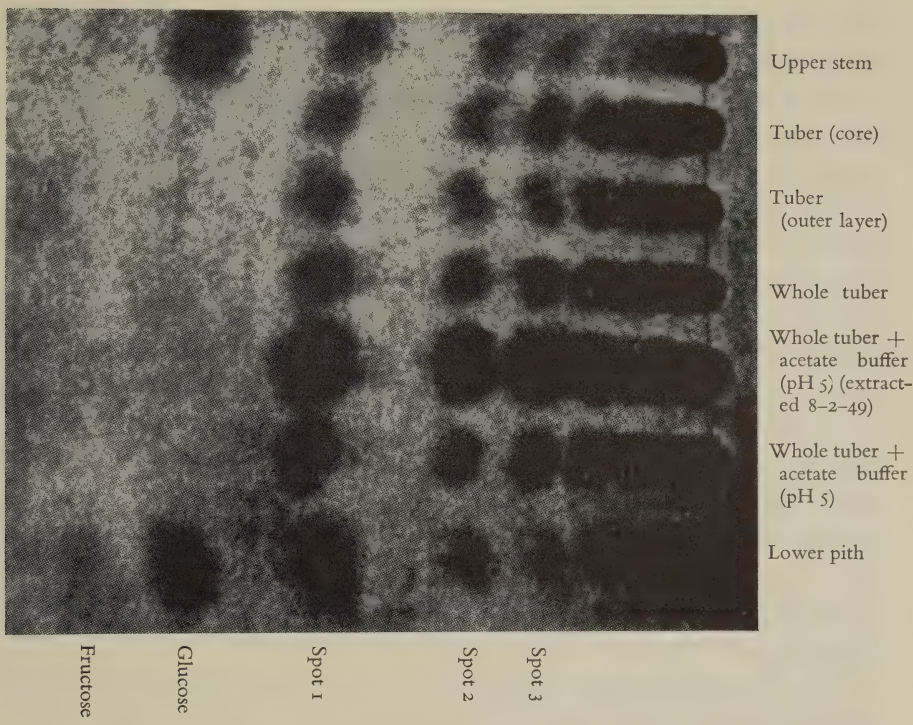


Fig. 5. One-dimensional paper chromatograms of extracts from the Jerusalem artichoke (*Helianthus tuberosus* L.) showing fructosan-like oligosaccharides. (J. S. D. Bacon and J. Edelman, *Biochem. J.*, 48 (1951) 114.)

cules. However, partition chromatography is admirably adapted to effecting separations within homologous series, and the use of non-aqueous or multicomponent two-phase liquid systems combined with the various procedures for determining which phase in the chromatogram shall be stationary have greatly increased the scope of the method for dealing with lipides in recent years. I would like here to pay tribute to the usefulness of gas–liquid chromatography as developed by James and Martin for analysing the lower fatty acids, which are the main nutrients absorbed by the ruminant animal from its intestinal tract. The possibility of rapid simultaneous analysis of all members of this group will help to increase our understanding of important problems in the physiology of these animals.

Partition chromatography has now been used for studying a wide variety of other substances of biological interest. Time prevents detailed discussion,

but mention should be made of alkaloids, glycosides, amines, alcohols, aldehydes, ketones, carboxylic acids and their derivatives, tannins and a wide variety of natural pigments, as well as many of the highly assorted chemical substances included in the groups coenzymes, vitamins, hormones and antibiotics.

Before leaving the biochemical uses of partition chromatography I would like to give an illustration of how the method can be useful in pharmacological and similar studies. R. A. Peters and colleagues have studied in detail the toxic effects of fluoroacetic acid on living tissues, and partition-chromatographic methods were used to show that fluoroacetic acid is transformed into another substance which acts directly on an enzyme system in the tricarboxylic acid cycle. The chromatographic behaviour further gave preliminary evidence of the chemical nature of the transformation compound (a fluorinated tricarboxylic acid) before this had been isolated in quantities suitable for chemical study. The fact that the method works just as well with ultramicro-quantities as on the ordinary scale implies that it has an important future for studying the mode of action and fate of substances of very high biological activity.

Outside biochemistry, it is probably the technological applications of partition chromatography that are most important. However, the method, particularly using cellulose, has proved useful for a wide variety of separations of metals and inorganic ions, and the fact that it can be used to handle minute quantities of carrier-free radioactive isotopes gives it considerable scientific importance. However, the main importance of the method seems to be towards making easier, cheaper and quicker the various analytical operations used in industrial research and routine control of production. The applications in metallurgy are obviously considerable, and the method is useful for control of intermediates in organic-chemical industry generally, whether concerned with dyestuffs, drugs, explosives, plastic materials, or other products. The gas–liquid chromatogram, representing fractional distillation pushed to its logical conclusion, obviously has an important future rôle in industrial research and control wherever mixtures of volatile compounds have to be handled, as in the fermentation, tar-distilling and petroleum industries. Here the method seems likely to compete with mass spectrometry. Except possibly with the most expensive drugs, partition chromatography has had little use as a method for actual production, on account of the large bulk of apparatus, chromatographic materials and solvents in relation to the substances being separated.

Partition chromatography facilitates in many obvious ways the work of those engaged in the control of foods and drugs, in clinical laboratories and generally in the safeguarding of the public health, while making more difficult the work of adulterators and other criminals. The dietitian and food technologist, as well as the pure biochemist, also gain by being able more cheaply and quickly to assay chemically a wide variety of foodstuffs for their essential constituents and to be able to ascertain the effects on these of processing, cooking and so forth. A striking recent use of the method was the isolation from flour processed with nitrogen trichloride (« Agene » process) of a toxic derivative of methionine, which was shown to be responsible for the « running fits » induced in dogs fed on this flour. For some reason governmental action towards prohibiting this treatment first occurred in our country when this chemical evidence became available. Irrespective of this particular case, one cannot help, in these days of increasing sophistication of foodstuffs, feeling proud at having aided the analyst in his increasingly complicated duties.

There are two new trends in analytical work which I must deal with briefly, although they are not essentially connected with partition chromatography. However, the fact that partition chromatography permits the separation on paper of such a wide variety of substances has made it of decisive importance for this work.

The first is the use of radioactive isotopes as tracers. Radioactive chromatographic zones are particularly conveniently studied on paper, either by scanning with a counter or by radioautographic contact printing. The use of radioactive tracers in combination with paper chromatography has enormously helped in discovering metabolic pathways in living organisms. The first studies of this kind were done with ^{131}I by R. M. Fink, C. E. Dent and K. Fink (1947). Since then a number of workers have elucidated the metabolism of this element in the thyroid gland, culminating with the recent observation by J. Gross and R. V. Pitt Rivers that triiodothyronine and not thyroxine may be the physiologically active form of the thyroid hormone. ^{14}C has of course a wider field of application in biochemistry; its most striking use so far in conjunction with chromatography has been the use by M. Calvin and colleagues of radioautographic printing of two-dimensional filter-paper chromatograms to follow the fate of ^{14}C from $^{14}CO_2$ during different time intervals after its assimilation by the photosynthesizing green plant. These observations, although not leading to any simple picture of the photosynthetic process, have provided a wealth of facts sufficient to disprove all

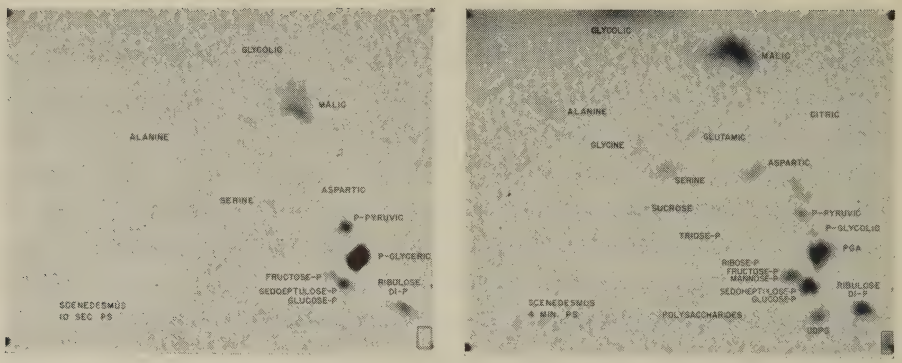


Fig. 6. Radioautographs of two-dimensiona paper-chromatograms of extracts of *Scenedesmus* after 10 sec (*left*) and 4 min (*right*) photosynthetic assimilation of carbon dioxide labelled with ^{14}C. (*Courtesy of Prof. M. Calvin.*)

detailed theories of photosynthesis previously advanced and they suggest new directions for future experiments (Fig. 6).

Mention should also be made of the ingenious use of radioactive elements in a substituent grouping for extending the isotope dilution method of analysis to the ultramicro scale. A. S. Keston, S. Udenfriend and M. Levy substituted the unknown amino-acid sample with *p*-iodobenzenesulphonyl groups having radioactive I and added a known quantity of the synthetic derivative having radioactive S. Differential counting of ^{35}S and ^{131}I in a single spot on a paper chromatogram thus permitted accurate analysis for μg quantities of amino acid. The ratio of the activities at different regions of the spot provided rather rigorous evidence of the chemical identity of the compounds prepared.

The second special application of paper chromatograms is for locating substances affecting the growth of micro-organisms « bioautographically ». Following the work of R. R. Goodall and A. A. Levi with the penicillins this procedure has been widely used in studying antibiotics and growth factors for normal and mutant organisms. An agar plate is impregnated with the test organism, and the developed paper chromatogram is laid on it. Diffusion of substances from paper to plate is allowed to occur at low temperature for a suitable time. The plate is then incubated and the different active substances are identified as zones of stimulated or inhibited growth (Fig. 7). Such procedures have helped quickly to reveal the complicated variety of chemical substances often responsible for a single biological effect. Striking examples are provided by recent research in the vitamin B_{12} and coenzyme A groups.

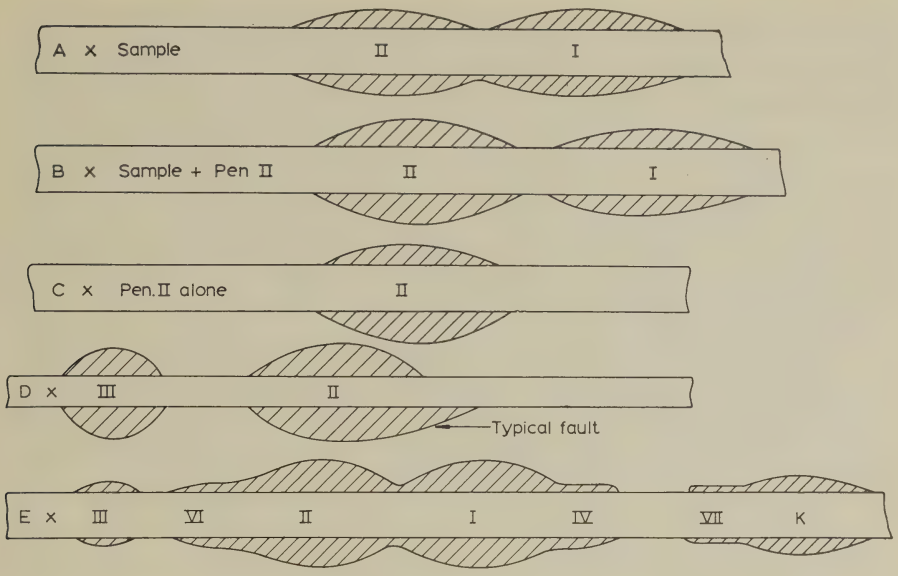


Fig. 7. Bioautographic detection in agar plates of zones of inhibition of bacterial growth due to penicillins separated on paper-strip chromatograms. (R. R. Goodall and A. A. Levi, *Analyst*, 72 (1947) 277.)

What I have said gives some idea of the scope and field of application of partition chromatography. But it does less than justice to the hundreds of workers who have helped in the past decade to explore rather thoroughly the possibilities of the method. One should bear in mind also the parallel development of bulk countercurrent distribution between two liquid phases for which L. C. Craig and colleagues have perfected the technique. While these procedures lack either the simplicity or else the resolving power of partition chromatograms, they will conveniently handle greater quantities of material and the form of the distribution curve can give a rather rigorous test of homogeneity. Craig's methods have another important advantage which I will deal with later.

It must further be remembered that these partition methods are only one group in a wide family of refined physicochemical separation procedures developed during the present century and especially during the past decade. Adsorption chromatography, using M. S. Tsvett's elution development and such agents as alumina and charcoal, is a very powerful method giving useful separations, in many cases where partition chromatography is not very effective. Its field of application has been considerably extended by the displace-

ment and carrier-displacement development procedures originated by A. Tiselius and colleagues. Further, it is only in recent years that the potentialities of the newer ion-exchange materials for chromatography have at all fully been realized. And leaving aside chromatography altogether, there are a variety of electrical-transport procedures which will effect refined separations of charged substances. With all these methods at his disposal it has become, for the analyst, essentially a routine matter to separate any pair of substances of molecular weight lower than, say, 500.

But biochemists, although they value these recently acquired powers, are not content with them. They are deeply concerned with purifying substances of still higher molecular weight and are finding the methods at present available to be increasingly inadequate to their requirements. Efforts to use partition chromatography for separating larger molecules have often been unsuccessful. Two main reasons for this are becoming apparent.

First, the larger molecules have either very high or very low partition coefficients in the simple two-phase two-component systems. The theoretical reason for this was clearly expounded by J. N. Brønsted in 1931. Consequently one must use more complicated, multicomponent systems with the larger molecules. This involves many trials and much error. Second, the solids most used hitherto for partition chromatography (cellulose, starch, silica gel, etc.) have pores of molecular dimensions which will not admit the larger molecules. Kieselguhr, where the spicules entangle droplets of liquid phase, is largely free from this defect, and it is surely no accident that the most successful separations of larger molecules by partition chromatography have been those of some simpler proteins by A. J. P. Martin and R. R. Porter using kieselguhr. The bulk distribution techniques of Craig and colleagues have also been successful with larger peptides and some smaller proteins. Here even the interface between the two liquid phases as well as the kieselguhr surface can very largely be eliminated as sites for adsorption.

However, despite these potentialities, it seems to me that the liquid–liquid methods are likely, when we come to these larger molecules, to be less easy of extension than the other techniques which I have mentioned. And there are also possibilities for new techniques based on diffusion, molecular-sieve effects and so forth. Irrespective, though, of the future importance to the biochemist of partition chromatography as an analytical technique, I think it will be found in future years to have had an influence on biochemistry which is not very widely appreciated at present, when so many biochemists are using it simply as an analytical method for skimming the cream of their

science. And here of course the method *is* giving to the biochemist something akin to what the microscope gave to earlier biologists, permitting a recognition of that characteristic interplay of diversity and uniformity that seems to be found in living things whether the student's measure is graduated in cm, μ or Å. But each R_F value or partition coefficient determined in each solvent system gives a new fact (or confirms a prediction) about the secondary-valence interactions of certain small molecules, and hence adds to the general body of knowledge of intermolecular forces. When the effects with small molecules are better understood we shall be much better able to understand from the structure of the larger molecules what is their function in terms of such intermolecular forces. Then chemistry really will begin to merge with biology as closely as it has already merged with physics. Partition chromatography may well be remembered for this contribution long after it is quite obsolete as an analytical method.

Biography

Richard Laurence Millington Synge was born at Liverpool on October 28th, 1914, as the son of Laurence Millington Synge, of Liverpool Stock Exchange, and Katharine Charlotte Swan. In 1928 he went to Winchester College, where he studied mainly classics until 1931, thereafter natural science. In 1933 he entered Trinity College, University of Cambridge and studied physics, chemistry and physiology for Part I of the Natural Sciences Tripos (1935) and biochemistry for Part II (1936). During 1936–1939 he was a research student under supervision of Mr. N. W. Pirie in the University Biochemical Laboratory headed by Sir Frederick G. Hopkins, and during 1939–1941 at the Wool Industries Research Association at Leeds. He obtained his Ph.D. degree at Cambridge in 1941. In the same year, he joined the staff of the Wool Industries Research Association at Leeds and in 1943 that of the Lister Institute of Preventive Medicine, London, in the Biochemistry Department under W. T. J. Morgan. Since 1948, he has been Head of the Department of Protein Chemistry at the Rowett Research Institute at Bucksburn, Aberdeen.

The circumstances of his work up to 1945, including the collaborative work on partition chromatography and related topics, are described in the accompanying Nobel Lectures by A. J. P. Martin and himself. They gave the first demonstration of partition chromatography to the Biochemical Society at its meeting at the National Institute for Medical Research, London, on June 7th, 1941 [*Chem. Ind. (Lond.)*, 19 (1941) 487], the first published description appearing in the *Biochemical Journal*, 35 (1941) 1358.

Since 1945 Dr. Synge has been mainly interested in analytical problems concerning the larger peptide molecules, as antibiotics and as intermediates in protein metabolism. From 1942 to 1948 he worked almost exclusively with the antibiotic peptides of the gramicidin group. In 1946–1947 he spent eight months with Professor Tiselius at Uppsala, studying the application of his adsorption methods to these compounds.

At the Rowett Research Institute, directed by D. P. Cuthbertson, he has been particularly concerned with the digestion of proteins by the ruminant

animal and its associated micro-organisms, with peptides, proteins and other components of plant material, and with physico-chemical methods for the purification of intermediates in the metabolism of proteins. Work begun about 1950 with D. L. Mould and A. Tiselius on electrokinetic ultrafiltration of various polysaccharides has been developed in a number of directions to take advantage of molecular-sieve effects, especially in the presence of hydrogen-bond breaking solvents.

In 1958–1959, he spent a year at Ruakura Animal Research Station, Hamilton, New Zealand, working with E. P. White on isolation of the toxic fungal component sporidesmin.

Dr. Synge was made a Fellow of the Royal Society in 1950 and of the Royal Institute of Chemistry in 1952. He is an honorary member of the American Society of Biological Chemists.

In 1943 he married Ann Stephen, daughter of the late Adrian and Karin Stephen, psychoanalysts. They have four daughters and three sons, in order of decreasing age: Jane, Elizabeth, Matthew Millington, Patrick Millington, Alexander Millington, Charlotte, and Mary.

Chemistry 1953

HERMANN STAUDINGER

«for his discoveries in the field of macromolecular chemistry»

Chemistry 1953

Presentation Speech by Professor A. Fredga, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Royal Highnesses, Ladies and Gentlemen.

«Even the ancient Greeks...» is a frequent preamble to the survey of a historical event and the hearer sees a vision of frightening profundity. I should like today to begin with Democritus of Abdera who formulated the first atomistic conception of the world and thus created the first atomic concept. However, the meaning of this concept did not become more closely defined until about 1800 by the Englishman Dalton who assumed that each element had its specific type of mutually identical atoms. In the formation of a chemical compound a number of atoms of two or more elements are linked together by chemical bond forces into particles for which the Italian Avogadro introduced the name molecules. In the second half of last century through the work of the German Kekulé and the Dutchman Van 't Hoff, knowledge was gained of important principles in the architecture of the molecules. The relative positions of the atoms have been determined to a certain extent; they link one another together to form chains, simple or branched, or to form more complex structures. Both molecules and atoms, however, were purely hypothetical concepts at that time; not until the turn of the century was definite proof of their actual existence forthcoming, and so it became possible to determine their actual size and mass. As expected they were found to be very small. The number of molecules in one litre of water is expressed by a number containing 26 digits.

It was often wondered how many atoms could be combined in one molecule, to what degree this compression of matter could be taken, to use the phrase with which the situation was expressed by a leading research worker of the day. The fact that the atoms in the molecule are held together by chemical bond forces is closely related to the question of the strength of the chemical bond, of which only little was known. On the one hand it was known that the molecules of apparently very stable compounds could readily be split by electrolytic dissociation, on the other hand there was a dawning realization that chemical forces had a profound bearing on the structure of solid crystals. Molecules with one to two hundred atoms had been built

in stages and this fact was considered remarkable. There was an eagerness to experiment, to advance further, but it was believed that little more could be achieved in this way.

In the early 1920's Professor Staudinger expressed the view that a molecule could be very large, almost arbitrarily large in fact, that such macromolecules could very easily, sometimes with apparent spontaneity, be formed of 10,000 or 100,000 atoms and that the particles of colloidal solutions were in many cases actual molecules of this type. I shall attempt to reproduce his trend of argument.

In organic chemistry it not infrequently occurs that sparingly soluble or insoluble resinous or pitch-like masses are obtained instead of the product expected; occasionally a change of this type occurs without visible cause. All the indications are that in some way the molecules become joined together. Such products were usually designated high polymer or high molecular substances but for many reasons there was a desire to regard the phenomenon as a physical one. On the other hand numerous cases were known where a small number of molecules, perhaps two or three, united to yield a larger molecule with a ring structure. Professor Staudinger pointed out that the formation of high molecular substances occurs where ring closure is expected in principle but is hindered for geometric reasons or, in other words, in cases where the ends of the atomic chains only meet with difficulty. Here, however, the chain ends must link up with other molecules which in turn capture new molecules and so the chain grows until the process is interrupted by some external circumstance, perhaps because the material is exhausted. The high molecular products were thus alleged to consist of chains formed in this way and since there can scarcely be any doubt that the ring molecules just referred to have been created by means of normal chemical bond forces, such must also be the case with the chains.

This argument, which strikes us nowadays as completely obvious, was very strange in the early 1920's, running partly counter to the spirit of the period, and the next ten years were charged with controversy. It was theoretically very difficult and practically very laborious to find decisive evidence or counter-evidence. For the time being it was impossible to determine the molecular weights of the magnitudes involved here but it was considered that the new theory placed impossible demands on the strength of the chemical bond forces. Concepts and definitions had to be revised, including the chemical compound concept. Categories of compounds had to be recognized in which the molecules are not completely identical. The high

molecular compounds consist of chain molecules constructed according to a common pattern and often with a characteristic average length but the length of the individual chains depends upon arbitrary circumstances. The new theory was not universally recognized until the 1930's.

The macromolecular theory had then already been adopted in technology. People learned to use the strong giant molecules for the manufacture of what are nowadays commonly termed plastics. Isolated products of this type had been known previously but now theoretical principles for further work, with almost limitless possibilities for varying the properties of the material according to different requirements were available, and so in the 1930's and 1940's a powerful growth came about in this sector. We are aware that in many respects this development has laid its imprint on the modern material culture; it is indeed stated that we are living in the age of plastics. For pure science too, however, the macromolecular theory has been of the greatest significance.

An impressive number of workers have been active in the macromolecular field during the last decennia. Professor Staudinger has not been involved directly in the technical and industrial development but without his energetic and bold pioneering work this development would scarcely be conceivable.

Professor Staudinger. More than thirty years ago you expressed the view that a chemical molecule can attain an almost arbitrary size and that such macromolecules are of great importance in our world. Your view was based on logical reasoning. You drew attention to the fact that what are termed high polymers are formed when for some reason or another an anticipated ring closure fails to occur. You thus submitted an argument which an organic chemist cannot ignore. Moreover, in extensive and painstaking series of studies you have provided experimental proof.

It is no secret that for a long time many colleagues rejected your views which some of them even regarded as abderitic. This was understandable perhaps. In the world of high polymers almost everything was new and untried. Long standing, established concepts had to be revised or new ones created. The development of the macromolecular science does not present a picture of a peaceful idyll.

As time passed, the conflicts vanished and the controversies were stilled. Unity has been achieved on the major issues and the importance of your pioneering work has become more and more apparent. In recognition of

your services to the natural science and the material culture made possible by your discoveries in the field of high molecular compounds, the Royal Swedish Academy of Sciences has resolved to award you the Nobel Prize for this year. I congratulate you on behalf of the Academy and would ask you to receive the Nobel Prize from the hands of His Majesty the King.

Hermann Staudinger

Macromolecular chemistry

Nobel Lecture, December 11, 1953

Macromolecular chemistry is the youngest branch of organic chemistry and as such has experienced the honour of the award of the Nobel Prize for Chemistry. I sincerely hope that this great distinction will be the means whereby macromolecular chemistry will undergo further fruitful development.

Some few months after I had the opportunity of speaking in this auditorium on the development of macromolecular chemistry into a new branch of organic chemistry at the International Congress for Pure and Applied Chemistry[1], it is today my duty to describe to you the characteristic features of macromolecular chemistry and demonstrate the new features which it introduces into organic chemistry.

The macromolecular compounds include the most important substances occurring in nature such as proteins, enzymes, the nucleic acids, besides the polysaccharides such as cellulose, starch and pectins, as well as rubber, and lastly the large number of new, fully synthetic plastics and artificial fibres. Macromolecular chemistry is very important both for technology and for biology.

In common with all organic compounds, the structure of the organic macromolecular compounds (inorganic macromolecular compounds are not discussed in the following) involves in addition to carbon atoms, chiefly hydrogen, oxygen, and nitrogen atoms which in accordance with the laws of Kekulé's structural theory are bound by chief valences[2]. The only difference between macromolecules and the small molecules of low molecular substances is one of structural size. If it is desired to lay down a boundary between macromolecular and low molecular compounds – there are of course transitions linking the two groups – the substances with a molecular weight greater than ten thousand, i.e. the molecules of which consist of one thousand and more atoms, may be classified as macromolecular. Beyond roughly this size, characteristic macromolecular properties occur. So far no upper limits can be given for the size of the macromolecules. Macromolecular compounds with a molecular weight of several millions are known,

i.e. compounds in which one million and more atoms form the macromolecules in the manner prescribed by Kekulé's structural theory.

In recent decennia the field of macromolecular chemistry has been the scene of very intensive scientific and technical activity. I personally have been concerned[3] with macromolecular chemistry since 1920 initially at the Federal Institute of Technology in Zurich. Since my move to the chemical laboratory in Freiburg University I have devoted myself entirely to extending this field which, since my retirement, has been further studied in a special research institute in Freiburg.

In this work I have been assisted by a number of first-class colleagues who have published valuable research on this field. Here I should like to mention the oldest of them, Signer[4], now in Bern, whose support during the discussions on the structure of these compounds in the 1920's was very valuable and who introduced the flow birefringence method for studying the particle shape of macromolecular substances. In addition Schulz, now in Mainz, extended the physico-chemical studies in the last two decennia, especially the molecular weight determination of macromolecular substances[5]. He also worked on the kinetics involved in the formation of macromolecular substances[6]. Kern[7], now in Mainz, studied the behaviour of polyelectrolytes. Husemann[8], Freiburg i. Br., studied polysaccharides, i.e. starch, wood polysaccharides and, in conjunction with Schulz, the fine structure of cellulose[9]. Kohlschütter[10], now in Darmstadt, investigated topochemical reactions using polyoxymethylenes. Batzer[11] has in recent years successfully continued Carothers' work on polyesters. Hengstenberg[12], Sauter[13] and Plötze[14] carried out X-ray studies at different periods. Staudinger (M.)[15] conducted morphological studies of macromolecular substances, and introduced light, ultraviolet and electron microscopy – and for some time now, phase contrast microscopy as well – into macromolecular chemistry. In addition, over the last 25 years in which almost 400 publications have appeared, she has collaborated in these and a series of books. She is the originator in particular of new considerations in respect of the relations between macromolecular chemistry and biology[16].

I cannot mention here the names of all the individual assistants and graduate students but today I remember with gratitude the assistance of all those colleagues who have participated in expanding this field.

Since, as I explained, macromolecular compounds are built up according to the laws of Kekulé's structural theory in exactly the same manner as the low-molecular organic compounds, i.e. they are genuine organic com-

pounds but with particularly large molecules, the question arises whether there is any need at all to classify macromolecular chemistry as a new field of organic chemistry. It has been found, however, that owing to the size of the macromolecules, a whole series of new problems do actually arise here so that in many respects macromolecular chemistry differs substantially from low molecular chemistry.

Even the classification of the macromolecular compounds is based on other criteria than in low molecular chemistry: the naturally occurring macromolecular substances are conveniently treated separately from the fully synthetic compounds (Table 1).

Table 1. Classification of macromolecular substances.

I. Substances occurring in nature
1. Hydrocarbons – rubber, guttapercha, balata.
2. Polysaccharides – celluloses, starches, glycogens, mannans, pectins, polyuronic acids, chitines.
3. Polynucleotides (nucleic acids).
4. Proteins and enzymes.
5. Lignins and tans (transition from low- to macromolecular substances).

II. Conversion products of natural substances
Vulcanized rubber, rayon, cellophane, cellulose nitrate, leather, lanital, galalith, etc.

III. Synthetic materials
Plastics (polyplastics) formed by
polymerization – buna, polystyrene, polymethacrylic ester.
polycondensation – bakelite, nylon, Perlon, Terylene.
polyaddition – polyurethane.

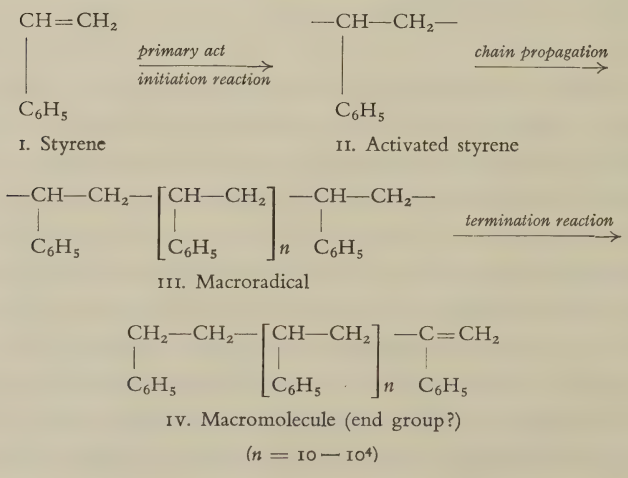
For the low molecular compounds such a division is unnecessary and irrelevant; the low molecular substances occurring in nature can largely be manufactured synthetically and these synthetic compounds are indistinguishable from the natural products. The situation is otherwise for the macromolecular compounds. It has so far proved impossible to build a macromolecular natural substance by a clear, stepwise synthesis from low molecular compounds. Thus synthetic polyisoprene is not identical with, say, natural rubber but has an essentially different constitution and hence other physical properties as well. In deriving macromolecular natural substances from vegetable and animal material the original macromolecules are in many cases modified to a greater or lesser extent by isolation and purification, and thus

the macromolecules examined are not the same as those formed by Nature. Problems of this type prevail for instance in the production of cellulose, starch, and many proteins. Low molecular products on the contrary can be isolated from natural products without modification. It is furthermore possible that Nature creates macromolecules of uniform size but positive proof of this fact has so far been found only in a few cases. In 1926 for example Svedberg[17] made the astounding discovery that a number of respiratory proteins are monodisperse, i.e. that they are composed of uniformly large macromolecules. Recently a whole series of enzymes and hormone proteins have been crystallized so that here too the occurrence of natural products with uniformly large macromolecules is very probable[18].

Against that the technically manufactured transformation products of natural macromolecular substances which can be termed semi-synthetic products are invariably polymolecular mixtures since the original macromolecules are broken down to a greater or lesser extent during processing. In all fully synthetic products, i.e. in plastics and fibres, there are also inseparable mixtures of polymer homologues. It is thus impossible to prepare completely uniform products by synthesis as was formerly considered necessary by the organic chemist to elucidate the constitution of organic compounds.

Macromolecular products are created in various ways. The first to deserve mention is polymerization, constituting a particular chain reaction such as is unknown in low molecular chemistry[19] (Formula 1). Owing to the

Formula 1. Polymerization of styrene.



I. Styrene

II. Activated styrene

III. Macroradical

IV. Macromolecule (end group?)

($n = 10 - 10^4$)

technical importance of the polymerisates, e.g. polystyrenes, polymethacrylic esters and polyvinyl chlorides, this reaction has been studied very thoroughly with reference to the kinetics and the influence of catalysts on the course of the polymerization[20].

In addition, macromolecular substances can be produced by polycondensation, a process which has long been known to technology and the one whereby Baekeland secured the phenoplasts which are of unusual technical importance. This field was then further studied by Carothers[21] and led to such technically valuable products as nylon (Formula 2).

Formula 2. Polyamides.

1. From hexamethylenediamine + adipic acid → nylon (W. H. Carothers)

$$(n+1)NH_2(CH_2)_6NH_2 + (n+1)HOOC(CH_2)_4COOH \rightarrow$$
$$NH_2(CH_2)_6NH[CO(CH_2)_4CONH(CH_2)_6NH]_nCO(CH_2)_4COOH + (n+1)H_2O$$
$$(n = 100 - 250)$$

2. From ε-aminocaproic acid → Perlon (P. Schlack)

$$NH_2(CH_2)_5COOH + (n+1)NH_2(CH_2)_5COOH \rightarrow$$
$$NH_2(CH_2)_5CO[NH(CH_2)_5CO]_nNH(CH_2)_5COOH + (n+1)H_2O$$

A further technique to produce macromolecular compounds evolved by O. Bayer[22] is polyaddition with diisocyanates (Formula 3).

Formula 3. Polyurethanes.

e.g. from hexamethylenediisocyanate + 1,4-butanediol

$$(n)\ O{=}C{=}N(CH_2)_6N{=}C{=}O + (n+1)HO(CH_2)_4OH \rightarrow$$
$$HO(CH_2)_4O[OCNH(CH_2)_6NHCOO(CH_2)_4O]_nH$$
$$(n = 20 - 100)$$

In the most favourable case therefore, in the manufacture of fully synthetic products, uniform polymeric compounds are formed, i.e. mixtures of filamentary molecules of equal constitution but different length. Very recently, research conducted by Melville[23] and others showed that polymerization processes are frequently more complex than had formerly been assumed so that the resultant products are mixtures of polymer isomers.

Since most natural macromolecular substances as studied are polymolecular mixtures in common with all synthetic macromolecular substances, a

macromolecular substance must be identified by different criteria than a pure low molecular substance. Ostwald (Wi.)[24], in his Analytical Chemistry, has pointed out that the task of the analytical chemist is facilitated by the fact that the agreement of just a few properties of two substances is sufficient for regarding them as identical. This principle applies only for low molecular compounds in which, owing to the small size of the molecules or ions, relatively large differences in the properties occur, i.e. they differ abruptly from one compound to another. The situation is different for macromolecular compounds; a plastic such as, say, a polystyrene can be consistent with another polystyrene in a number of essential properties and still differ in composition. It is moreover also impossible to characterize and identify macromolecular substances by the melting point and by the mixed melting-point test, a technique which has contributed greatly to the rapid successes in the identification of natural substances in low molecular chemistry.

Initially identifying the constitution of macromolecular compounds seemed to be further complicated by the fact that they do not yield normal monodisperse solutions but that – if they dissolve at all – they mostly form polydisperse, colloidal solutions. The procedure for elucidating the constitution of organic compounds can again be referred to here: It consists in dissolving the particular compound after elementary analysis and then determining the size and finally the intrinsic structure of the dissolved particles by further studies. With completely insoluble substances occurring as a single aggregate only[25], details of the type of bond in the smaller groups in their molecules can in many cases be disclosed by decomposition; but the molecular weight of such a substance is indeterminable. Against that, however, it may not be inferred from the insolubility of a substance alone that it has a particularly

Table 2. New classification of disperse systems by the number of atoms.

Increasing degree of dispersion.
Coarse dispersions The particles are composed of more than 10^9 atoms, do not pass through filter paper and can be made visible microscopically. They are polydisperse.
Colloids The particles are composed of 10^3 to 10^9 atoms, pass through filter paper, cannot be resolved microscopically and cannot be dialysed. They are polydisperse or monodisperse.
Low molecular dispersions The particles are composed of 2 to 10^3 atoms, cannot be resolved microscopically, diffuse and dialyse readily. The molecules or ions of uniform substances are identical in structure and size.

high molecular weight, a fact which emerged during studies on aminoplasts[26] in the laboratory at Freiburg.

When considering the various modes of dispersion of organic substances in a liquid, it is expedient to indicate the size of the dispersed particles by the number of atoms they contain, the convention[27] adopted in Table 2. The particles of the low molecular organic compounds thus contain up to a maximum of 10^3 atoms and as a rule are identical with the molecules. The molecule concept is defined here in the same way as in low molecular chemistry: a molecule is the smallest particle in which all the atoms are linked by chief valences[28]. It can readily be ascertained whether such particles contain not molecules but associations or double molecules, as applies with e.g. solutions of fatty acids in benzene.

The particles of colloidal size which are composed of 10^3 to 10^9 atoms can have a much more varied structure than the particles made up of fewer atoms (Table 3).

It follows from Table 3 that for one thing lyophobic colloids can occur which formerly roused special interest when Ostwald (Wo.)[29] drew attention to the «field of neglected dimensions» and pointed out that every substance can be broken down into particles of colloid size by appropriate

Table 3. Classification of colloidal solutions of organic substances.

	Behaviour to dispersion media	*Electrical charge*	*Viscosity of solutions*	*Example*
I. Dispersoid colloids				
(a) suspensoids	lyophobic	charged	low	sugar in benzene
(b) emulsoids	lyophobic	charged	low	oils in water
II. Micellar colloids (formed by aggregation of small molecules)	lyophilic	mostly charged	high	soaps in water
III. Molecular colloids (solutions of macromolecular substances)	lyophilic	charged or uncharged	low: spherocolloids	glycogens, proteins
			high: linear colloids	cellulose, proteins
IV. Macromolecular associations	lyophilic	charged or uncharged	low or high	proteins, simplexes, bio-colloids

dispersion. A further group of colloids are the lyophilic colloids to which belong on the one hand the micellar colloids and on the other the solutions of macromolecular substances. Since macromolecules are the size of colloid particles they can dissolve in no other way than colloidally, even if the solvent is changed, whereas with micellar colloids, e.g. the soaps, low molecular disperse solutions are also possible in certain solvents. The solutions of macromolecular substances may hence also be termed molecular colloids[30].

Surprisingly, one group of macromolecular compounds, the linear macromolecular substances, exhibit in many respects properties like those of typical micellar colloids. In particular there is great similarity here to the soaps, the colloidal nature of which was recognized at the beginning of the century through the work of Krafft[31], Zsigmondy[32], McBain[33], and others. Since the solutions of linear macromolecular substances, however, differ substantially from the solutions of low molecular substances, it can be understood why, a few decennia ago, the colloid particles in these solutions of macromolecular substances were assumed to have a micellar structure similar to those in aqueous soap solutions (Table 4).

Table 4. Properties of various substances.

Properties	*Low molecular substances*	*Linear macromolecular substances (e.g. rubber, starch, proteins)*	*Micellar colloids (e.g. soaps)*
Dissolve:	without swelling	with swelling	with swelling
Dissolved particles are:	monodisperse	polydisperse	polydisperse
Solution is:	Newtonian	non-Newtonian	non-Newtonian
1% solution is:	low viscous	high viscous	high viscous
Viscosity of solutions on standing:	constant	aging phenomena	aging phenomena
Dissolved particles are:	dialysable	undialysable	undialysable

Initially therefore, the efforts of the workers studying rubber, cellulose, starch, and proteins were aimed at determining the size of the molecules making up these micells. A number of workers such as Karrer[34], Bergmann[35], Pummerer[36] and Hess[37] assumed them to be small molecules whereas Meyer and Mark[38] held that these micells consisted of rather long chief valence chains.

Notwithstanding this similarity in the behaviour of micellar colloids and linear molecular colloids which derives from the elongated shape of the colloid particles in both cases, there is a profound difference between the two groups: the particles of the micellar colloids are loose aggregates of small molecules, in the case of the molecular colloids they are the macromolecules themselves.

The proof was obtained by the conventional methods of organic chemistry, i.e. by determination of the « macroradicals ». In this context the word « radical » is used in the meaning given to it by Liebig in his studies of the benzoyl radical. In many instances a polymeric compound can be transformed into derivatives of a different type without any change in the degree of polymerization of the compound in exactly the same way as small molecules can be transformed. A polymer compound can hence be transformed into polymer analogous derivatives, the transformation proving that all the basic molecules contained in the colloid particles of these polymeric compounds are linked together by chief valences, in other words the colloid particles are macromolecules. This proof becomes especially clear because out of a series of polymer homologues various ones can be transformed into polymer analogous derivatives. An explanation can be given with reference to cellulose.

The β-glucoside bond of the glucose radical in cellulose was established by the studies of Haworth[39] and Freudenberg[40]; furthermore Sponsler and Dore[41] demonstrated that the results of X-ray studies are consistent with the chain structure of cellulose. Subsequent studies then clarified the question whether the colloid particles in the solutions of celluloses and their derivatives have a macromolecular or micellar structure[42].

Table 5. Comparison of the molecular weights of decomposed cellulose triacetates as determined by the osmotic and end-groups methods, and the K_m-constants of these compounds.

Osmotic method		*End-groups method*		*Mean of*	$Z_\eta \cdot 10^3$ *in*	$K_m \cdot 10^4$
$\overline{M}$	$\overline{P}$	$\overline{M}$	$\overline{P}$	$\overline{P}$	*m-cresol*	
5,700	20	5,900	20	20	1.18	5.9
7,900	27	7,900	27	27	1.63	6.0
8,200	28	9,000	31	30	1.77	5.9
11,700	40	11,000	38	39	2.35	6.0
15,800	55	15,100	52	54	3.39	6.3
					Mean	6.0

In a polymer homologous series of decomposed low polymeric cellulose acetates, the molecular weight determined by the end groups agrees with that determined by the osmotic method, proof that unbranched filamentary molecules are present. The viscosity number Z_η of these compounds is proportional to the degree of polymerization as shown in Table 5.

The end-group molecular weight of higher polymer cellulose acetates can not be determined. The relations between the degree of polymerization determined by the osmotic method and the viscosity number Z_η are the same, however, as in the low molecular compounds appearing in Table 5, testifying that these high polymer cellulose acetates are dissolved in the macromolecular form and their chains are unbranched (Table 6).

Table 6. Determination of K_m-constants of higher polymeric cellulose triacetates in *m*-cresol.

$\overline{M}$	$\overline{P}$	$Z_\eta \cdot 10^2$	$K_m \cdot 10^4$
22,800	79	5.28	6.7
36,000	125	8.35	6.7
41,700	145	6.65	6.6
49,000	170	9.85	5.8
81,000	280	18.05	6.4
93,000	325	21.45	6.6
106,000	370	24.50	6.6
225,000	780	52.10	6.7
		Mean	6.5

With care, these cellulose acetates can be saponified to polymer analogous celluloses, so proving that both the cellulose acetates and the celluloses are present in solution in the macromolecular state[43] (Table 7).

Finally, with care being exercised, polymer homologous celluloses can be nitrated to polymer analogous cellulose nitrates with a mixture of nitric acid and phosphoric acid, so demonstrating the macromolecular structure of the cellulose nitrates[44] (Table 8).

The studies conducted at the Freiburg laboratory have frequently been concerned with determining macromolecular structure by polymer analogous transformations of this type. By that means it has been proved for further polysaccharides, i.e. starch[45], glycogen[46], mannan[47], and for a number of plastics that the colloid particles in their solutions are identical with the ma-

Table 7. Transformation of cellulose triacetates to polymer-analogous celluloses.

Acetates in m-cresol		*Celluloses in Schweizer's reagent*		Z_η-*acetates*	$K_m \cdot 10^4$
$Z_\eta \cdot 10^2$	*DP**	$Z_\eta \cdot 10^2$	*DP***	Z_η-*celluloses*	
3.4	54	2.8	56	1.21	5.0
18.0	285	15.3	305	1.17	5.3
21.4	340	17.6	350	1.22	5.2
60.4	960	47.9	955	1.26	5.0
74.4	1,180	58.3	1,165	1.27	5.0
104.5	1,660	84.0	1,680	1.24	5.1
			Mean	1.25	5.1

* Calculated from Z_η of column 1 with $K_m = 6.5 \cdot 10^{-4}$
** Calculated from Z_η of column 3 with $K_m = 5 \cdot 10^{-4}$

cromolecules. It is surprising that with these molecules, some of which are very large and complex, reactions can be conducted which are customary with molecules of lower molecular compounds. Such polymer analogous transformations hence provide an impressive instance of the stability of the macroradicals which is also of significance for the substances of living cells.

The question now arises of the origin of the frequent instability and changeability in the viscosity of macromolecular substances in solution. These « aging phenomena » arise because even slight amounts of low molecular substances are sufficient, chiefly in the presence of atmospheric oxygen,

Table 8. Transformation of celluloses into polymer-analogous cellulose nitrates.

Cellulose in Schweizer's reagent		*Cellulose nitrates in acetone*	
$Z_\eta \cdot 10^2$	*DP with* $K_m = 5 \cdot 10^{-4}$	$Z_\eta \cdot 10^2$	*DP with* $K_m = 11 \cdot 10^{-4}$
3.8	76	8.5	77
7.6	152	15.0	135
16.8	336	34.0	310
24.0	480	51.0	460
35.8	716	74.0	670
67.5	1,350	161	1,460
80.0	1,600	188	1,710
100	2,000	200	1,800
168	3,400	390	3,540

to decompose the linear macromolecules, leading to a profound change in the solution viscosity. This is shown in Table 9.

Table 9.

1 mol cellulose (DP 3,000)	+ 4 atoms oxygen	→ 2 mol cellulose (DP 1,500)
486,000 g cellulose (DP 3,000)	+ 64 g oxygen	→ 2 × 243,000 g cellulose (DP 1,500)
7.5 g cellulose (DP 3,000)	+ 1 mg oxygen	→ 2 × 3.75 g cellulose (DP 1,500)

Initially, as was the case for instance with rubber, these aging phenomena were particularly obscure because under certain conditions oxygen decomposes the filamentary molecules but can also link them together, this becoming apparent in an increase of viscosity and, should the linking of the filamentary molecules proceed further, it causes the soluble rubber to change into insoluble rubber. For this reason even polymer analogous transformations involving macromolecular substances are mostly to be conducted only under special experimental conditions since atmospheric oxygen for instance must be completely excluded. The tests are also complicated by the fact that the transformation products cannot be purified since all substances are polymolecular and reprecipitation together with purification would lead to a change in the composition of the polymolecular mixture. These complications do not exist where low molecular substances are involved.

With the most important group of macromolecular compounds, the proteins and nucleic acids, scarcely any polymer analogous transformations have so far been performed; here too it is difficult to produce polymer homologous series. The size and structure of the macromolecules have to be determined by different methods from those applied with the polysaccharides, rubber, and the plastics. The molecule concept as formulated for homopolar organic compounds is not applicable here without limitation since besides chief valences, very powerful secondary valences also participate in the structure of the particles.

The proof for the macromolecular structure of colloid particles is of fundamental importance since it signifies that these colloids cannot be considered in terms of the colloid doctrine as variable associations of small molecules but rather as macromolecules which must be treated by the methods of organic chemistry in common with molecules of low molecular substances[48].

There are still further profound differences between macromolecular and low molecular compounds: these are based primarily on the fact that the shape of the macromolecules affects the physical and chemical properties of the substances considerably more strongly than is the case with the low molecular compounds. Whereas, for example, normal nonane with elongated molecules, and tetraethylmethane with spherical molecules, both hydrocarbons having the composition C_9H_{20}, scarcely differ in terms of their properties, a glycogen in which 5,000 glucose radicals are linked to form a sphere has properties differing fundamentally from those of a cellulose in which the same number of glucose radicals are arranged in a long chain. This state of affairs led to the macromolecular substances being classified into two large groups, spheromacromolecular substances, i.e. substances with spherical molecules, and linear macromolecular substances with filamentary molecules. These two classes are, of course, bridged by transitions: a large number of natural and synthetic substances have heavily branched macromolecules, e.g. starch[45]. Table 10 indicates the significant bearing of the shape on the physical properties of natural macromolecular substances[49].

Table 10. Spherical and filamentary polysaccharides and proteins.

	Spherocolloids	*Linear colloids*
Polysaccharides	glycogen	cellulose, pectin
Proteins	ovalbumin, haemoglobin, lactoglobulin	collagen, myosin
Appearance in solid state	powdery	fibrous, tough
Swelling properties	fail to swell	swell considerably
Viscosity of 1% solution	low viscous solutions	high viscous gel solutions
Type of solutions	Newtonian	non-Newtonian, linear macromolecular or polyionic viscosity phenomena
Flow birefringence	none	increases with length of filamentary molecules
Osmotic pressure	in dilute solution obeys Van 't Hoff law	deviates from Van 't Hoff law
Diffusion	obeys Fick law	deviates from Fick law

In appearance, solubility, and in their further behaviour, macromolecular substances with spherocolloid molecules scarcely differ from low molecular substances; only the determination of the molecular weight proves that macromolecules are involved. The striking properties of macromolecular substances which, as listed in Table 4, caused a different type of micellar structure to be assigned to these compounds, occur only with the linear macromolecules; these include the natural and fully synthetic fibres, the rubber-elastic substances and many of the most important plastics. Linear macromolecular substances with long filamentary molecules behave in a manner alien to low molecular substances: inclusion and swelling phenomena, high solution viscosity, abnormal flow behaviour, etc. Since the constitution of these substances is known, their striking behaviour can be ascribed to the length of the filamentary molecules. A comparison of various examples of a polymer homologous series shows that as the filamentary molecules become longer, their properties change so profoundly that the earlier concepts which led to the conclusion that the units at the beginning and at the end had a completely dissimilar structure (Table 11) can be appreciated.

Table 11. Relation between physical properties and mean degree of polymerization (DP) of polymer homologous celluloses.

	DP	*Appearance*	*Fibre and film forming properties*	*Solubility and swellability in Schweizer's reagent*	*Viscosity of 1% solutions in Schweizer's reagent*
Oligosaccharides	1–10	powdery	triturable, forms neither fibres nor films	rapidly soluble, without swelling	low viscous sol solutions
β-Cellulose	10–100	powdery	partially solid, forms neither fibres nor films	dissolves without swelling	slightly viscous sol solutions
α-Cellulose	100–500	fibrous	solid, forms fibres and films	dissolves with swelling	viscous gel solutions
α-Cellulose	500–3000	long fibres	very solid and tough, very readily forms fibres and films	dissolves slowly with strong swelling	gel solutions of very high viscosity

It was therefore particularly difficult to study these linear macromolecular substances. Even to determine the molecular weight, for example, it was not possible to apply the customary physical techniques such as the osmotic, diffusion, or Svedberg ultracentrifuge methods until the abnormal behaviour of the solutions of these substances could be clarified[50].

The viscosity behaviour of the linear macromolecular substances is particularly striking. Whereas Einstein's law applies to the spheromacromolecular substances such as ovalbumin or glycogen, in the case of solutions of substances with filamentary molecules, the viscosity of solutions of equal concentration increases with the chain length, and with some of these substances, especially polysaccharides, there is a proportionality between the viscosity number Z_η and the chain length n or the degree of polymerization $\bar{P}$ of filamentary molecules. This viscosity number is the specific viscosity of a solution, i.e. the increase in viscosity caused by 1 g in one litre, or else its limit value. Since viscosity measurements are simple to perform, this method has been extensively adopted in technology to determine e.g. the degree of polymerization of celluloses and cellulose derivatives, as this parameter has a considerable bearing on the fibre properties as shown in Table 11. With other linear macromolecular substances such as e.g. the polyesters[11] and many polyvinyl derivatives, the relation between viscosity number Z_η and degree of polymerization is not as simple as with the polysaccharides, although there is a functional relation corresponding to an equation formulated by Kuhn[51] (Table 12).

Table 12. Viscosity relations.

$\eta_r = 1 + K \cdot \Phi$	Einstein, 1906
$\eta r - 1 = \eta_{sp}$	Staudinger and Heuer, 1930
$\lim_{c \to 0} \frac{\eta_{sp}}{c} = Z_\eta$	
$Z_\eta \cdot s = 0.0025$ (s = density)	e.g. valid for ovalbumin
$Z_\eta = K_m \cdot P$	Staudinger and Heuer, 1930, e.g. valid for cellulose and its derivatives
$Z_\eta = K_m \cdot P^x$	Kuhn, 1936, e.g. valid for polyesters

The reasons are as follows: the filamentary molecules of a linear macromolecular substance are usually elongated in the solid crystalline state. The length of the elementary units and hence the length of the filamentary mol-

ecules can be determined by X-ray examination, e.g. in the case of cellulose. On dissolution, owing to vibrations and intramolecular forces of attraction between the individual groups of the filamentary molecule, the latter becomes convolute to a greater or lesser extent depending on the temperature and also on the type of solvent. Thus the filamentary molecules in good solvents are more elongated than in poor solvents; in the former the viscosity number of a substance is higher than in a poor solvent. Kuhn[52] states that the entanglement of a filamentary molecule can be so pronounced in a poor solvent that the molecule assumes an almost spherical configuration such as has been experimentally determined with the polyisobutylenes[53]. A filamentary molecule would adopt that configuration in the gaseous state although in practice this state is unattainable, since the boiling point of all macromolecular substances lies far beyond the point at which they decompose.

Besides these reversible changes in shape which are governed by the nature of the filamentary molecule and the type of the solvent, chemical action also brings about irreversible changes in shape which permanently affect the physical properties of a linear-macromolecular substance. Thus it is common knowledge that owing to its long filamentary molecules, rubber yields highly viscous solutions with swelling. Against this, the solutions of chlorinated rubber, which is used as a paint, are of relatively low viscosity and therefore it was originally assumed that in the transformation from rubber to chlorinated rubber the long filamentary molecules were decomposed. Such is not the case as the degree of polymerization of chlorinated rubber is approximately the same as that of rubber[54]. Thus, when rubber is converted into chlorinated rubber, no decomposition takes place but rather a strong, chief valence cyclization which induces a permanent convolution of the filamentary molecules (Table 13).

Table 13. Polymer-analogous transformation of rubber and balata to chlorination products.

	$\overline{M}$	$\overline{P}$	Z_η	$K_m \cdot 10^4$
Rubber	182,000	2,700	0.46	1.7
Chlorinated rubber	410,000	2,600	0.077	0.30
Balata	99,000	1,450	0.188	1.3
Hydrochlorinated balata	135,000	1,400	0.088	0.63
Chlorinated balata	250,000	1,300	0.041	0.32

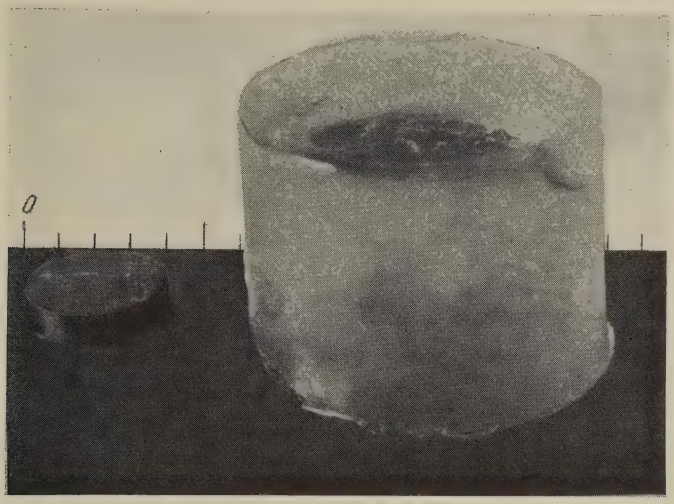


Fig. 1. Swelling of a piece of polystyrene with divinyl bonds. Before swelling (*left*). After swelling in benzene (*right*).

It is – as already mentioned – characteristic of the chemistry of macromolecular materials that the smallest amounts of substances are capable of modifying profoundly their physical properties. Hence a 0.0025% addition of divinyl benzene to styrene is sufficient during polymerization to link the chains of the polystyrene by divinyl benzene bonds[55]. Soluble polystyrene with its unlimited swelling capacity is thus transformed into a variety with limited swelling capacity which absorbs solvents by solvation and so swells without altering its shape (Fig. 1) and without being able to dissolve.

The replacement of a hydroxyl group in low molecular compounds by a methoxyl group alters the physical and, above all, the chemical behaviour of the compound considerably. The same applies also to macromolecular substances except that the percentage proportion of a methoxyl end group in the macromolecule can be so small that it readily escapes detection. Auerbach and Barschall[56] described two polyoxymethylenes: one is soluble in sodium hydroxide, the other insoluble, yet they are identical in appearance. The product soluble in sodium hydroxide is a polyoxymethylene dihydrate, the insoluble product a polyoxymethylene dimethylether[57] (Formula 4).

The slight percentage methoxyl end-group content thus blocks the decomposition of the chains by sodium hydroxide. This reagent can only at-

tack hydroxyl groups and so dissolve polyoxymethylene dihydrate. In biological processes as well, a slight percentage change in a macromolecule can bring about profound changes in the chemical and physiological behaviour of the macromolecular substance.

Formula 4. Formulae of polyoxymethylenes.

$$\underline{HO}—CH_2—O—(CH_2—O)_n—CH_2—\underline{OH}$$

α-polyoxymethylene: is decomposed by alkali

$$\underline{CH_3O}—CH_2—O—(CH_2—O)_n—CH_2—\underline{OCH_3}$$

γ-polyoxymethylene: is not decomposed by alkali

$(n = 1 - 200)$

Possibly the most important distinction between low molecular and macromolecular compounds is that the latter can exhibit properties which cannot be predicted even by a thorough study of the low molecular substances. This may be illustrated by comparing the organic molecules with buildings in which the bricks must be joined together systematically. With a few bricks it is impossible to erect a great variety of buildings; nevertheless, provided that 10,000 or 100,000 bricks are available it is quite possible to construct the most diverse buildings, viz. houses, halls, etc., the special construction of which cannot simply be predicted from the buildings comprising few bricks.

One such new type of behaviour of macromolecular substances are, for example, the swelling phenomena of the linear macromolecular substances caused by the solvation of the long filamentary molecules on the addition of solvent without immediately being able to go into solution. These swelling phenomena are complicated, as has been described, in that linking of the filamentary molecules induces a substance with unlimited swelling capacity to change into a substance of restricted swelling capacity[55], this also being a very potent factor with proteins. A further instance here are inclusion phenomena. Inclusion is an indirect swelling of linear macromolecular substances with liquids that are not solvents. They have mainly been studied in the case of cellulose, between the filamentary molecules of which inert liquids such as benzene, carbon tetrachloride, and others can be embedded. It is noteworthy that these liquids cannot be altogether eliminated from the linear macromolecular substance even under a high vacuum; they are held mechanically between the filamentary molecules of the substances. As a

Table 14. Amount of solvent included in mercerized cotton (DP 1,600) after drying for two days under high vacuum at 100°C.

Included liquids	*Amount included [%]*	*Number of glucose anhydride mols to one mol inclusion medium*	*Acetyl content [%] after one day's treatment with acetic acid anhydride-pyridine at 60°C*
A. Pure cellulose			
Water	0.0	—	0.3
Methanol	0.3	—	1.3
B. Inclusion cellulose			
Ethyl alcohol	2.4	12	21.2
Acetone	2.5	14	21.3
Pyridine	6.4	7	23.6
Cyclohexane	8.1	6	20.1
Benzene	3.4	14	19.1
Carbon tetrachloride	7.2	12	19.4

result of this inclusion, however, the reactivity of cellulose is appreciably raised[58] (Table 14).

A particularly significant phenomenon with linear macromolecular substances is a characteristic state of dissolution which is impossible with spheromacromolecular and low molecular substances: the state in which the filamentary molecules are completely solvated, i.e. essentially dissolved, but owing to their large bulk, their range of action, they have no free mobility. The term «gel solution» has been proposed for this characteristic state of dissolution which is intermediate between the normal state of dissolution and that of swelling[59]. The solutions of cellulose, cellulose derivatives and plastics as used technically are gel solutions.

It will be the task of macromolecular chemistry to examine further these new, characteristic properties which are governed by the size and configuration of the macromolecules since it will thus be possible to gain fresh insight into biological processes as well.

The existence of macromolecules and the steadily deepening knowledge of their properties have revealed the nature of the building units which the living cell requires to create matter[16]. The existence of macromolecules makes possible the vastly wide variation of the substances required; thus, for example, the very number of the isomers in protein molecules is practically infinite. Assuming a protein of molecular weight 100,000 and com-

posed of 20 different amino acids, the number of its isomers is 10^{1270}. The size of this number becomes clear when compared with the number of molecules of water present in the seas of the earth – a mere 10^{46} (Table 15).

Table 15.

Number of isomers of a protein having a molecular weight 10^5; the protein molecule composed of 50 molecules of each of 20 different amino acids.

Number of isomers: 10^{1270}.

For comparison the number of molecules of water in the seas of the earth:

Volume of the seas is about 1.3×10^{24} cm^3; 18 g water contain 6×10^{23} molecules.

The seas thus contain about 4×10^{46} molecules.

It is thus possible by isomerism alone to create an infinite number of substances. This number is increased still further by variation in the configuration of the macromolecules while they are being built, or else by the influence of their environment. So, each living organism can create its own nucleic acids, protein molecules, etc. This conclusion is necessary since every organism, and every human being too, differs chemically from another.

Associated with this boundless profusion of matter in the macromolecular sphere, in contrast, is a remarkable stability of the macromolecules, a stability which is governed by their structure as organic compounds in compliance with the laws of Kekulé's structural theory. That alone is capable of satisfying the «inconceivably strict demands on the integrity of the germ plasm» which are applied to the normal process of development of an organism[60]. This stability of the macromolecules, associated with their reactivity, supplies the living substance with the necessary basis for so specific a process as that of heredity.

The known facts of macromolecular chemistry show further that an individual macromolecule is still not «living», however large it is and however complex its structure. On the contrary, the term is relevant to a certain amount of substance comprising numerous macromolecules with the constituent small molecules combined together in strictly prescribed order, an «atomos» of living matter[16] which is indivisible without losing its «livingness». Living nature supplies the answer to the question how large such an «atomos» has to be in the form of the various germ cells, for nothing can be removed from such a cell, whether a spore or a fertilized ovum, otherwise no normal organism can be formed from it. One of the smallest units capable of self-propagation is, for example, a bacterial spore. The following estimate

Table 16. A bacterial spore of 0.124 μ diameter weighs 10^{-15} g (sp. gr. = 1) and after removal of 50% water content comprises $5 \cdot 10^7$ atoms.

Number of molecules when spore consists of						
30%	macromolecules	with 10^6	atoms	=	15	macromolecules
30%	„	„ 10^5	„	=	150	„
10%	„	„ 10^4	„	=	500	„
10%	molecules	„ 10^3	„	=	5,000	molecules
10%	„	„ 10^2	„	=	50,000	„
10%	„	„ 10	„	=	500,000	„

may afford an idea of what numbers of macromolecules and molecules are the necessary minimum for «livingness» here (Table 16).
In contrast to former opinions one of the smallest living units thus requires a quantity of substance.

In this way macromolecular chemistry appears today to fit between low molecular organic chemistry and cytology. It is the connecting link between them, growing systematically out of low molecular chemistry but, with the incomparably larger wealth of its chemical scope, forming living matter. In addition, over and above the quantitative laws of pure chemistry, macromolecular chemistry makes use of a number of qualitative correlations: those of shape and of the associated configurational scope, up to the level of the «atomos» of living substance, on which the game of Life ensues.

In the light of this new knowledge of macromolecular chemistry, the wonder of Life in its chemical aspect is revealed in the astounding abundance and masterly macromolecular architecture of living matter.

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Biography

Hermann Staudinger was born in Worms on the 23rd of March 1881. His father was Dr. Franz Staudinger.

Staudinger was educated in Worms, matriculated in 1899, and continued his studies first at the University of Halle, later at Darmstadt and Munich. He graduated at Halle in 1903 and qualified for inauguration as academic lecturer under Professor Thiele at Strasbourg University in spring 1907. In November 1907 he was appointed Professor of Organic Chemistry at the Institute of Chemistry of the Technische Hochschule in Karlsruhe. For fourteen years, from 1912, he was lecturer at the Eidgenössische Technische Hochschule in Zurich, and in 1926 followed the invitation of the University of Freiburg i. Br. to become Lecturer of Chemistry. In this city, he remained all through his further career. From 1940 onwards he held an additional appointment as Principal of the Research Institute for Macromolecular Chemistry. Staudinger resigned from his post as Principal of the Chemical Laboratories of the University in April 1951, and accepted the honorary appointment as Head of the State Research Institute for Macromolecular Chemistry, which he held until April 1956.

Staudinger was a prolific writer and the following books by him have been published: *Die Ketene* (Ketenes), published by Enke, Stuttgart, 1912; *Anleitung zur organischen qualitativen Analyse* (Introduction to organic qualitative analysis), published by Springer, Berlin, 1st edition 1923, 5th edition 1948, 6th edition 1955; *Tabellen zu den Vorlesungen über allgemeine und anorganische Chemie* (Tables for the lectures on general and inorganic chemistry), published by Braun, Karlsruhe, 1st edition 1927, 5th edition 1947; *Die hochmolekularen organischen Verbindungen, Kautschuk und Cellulose* (The high-molecular organic compounds, rubber and cellulose), published by Springer, Berlin, 1932; *Organische Kolloidchemie* (Organic colloid chemistry), published by Vieweg, Braunschweig, 1st edition 1940, 3rd edition 1950; *Fortschritte der Chemie, Physik und Technik der makromolekularen Stoffe* (Progress of the chemistry, physics and technique of the macromolecular substances), jointly with Professor Vieweg and Professor Röhrs, Volume I, 1939,

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Since September 1947 Staudinger has edited the periodical *Die makromolekulare Chemie* (Macromolecular chemistry), published by Dr. A. Hüthig, Heidelberg, and Wepf & Co., Basle.

In 1961 his book *Arbeitserinnerungen* (Working memoirs) appeared, published by Dr. A. Hüthig, Heidelberg.

Besides the books, Staudinger published a great number of scientific papers. Among these were fifty on ketenes, also works on oxalyl chloride, autoxidation, aliphatic diazo-compounds, explosions, insecticides, synthetic pepper and coffee aroma. Since 1920 he has written approximately 500 papers on macromolecular compounds, about 120 of these on cellulose, about 50 on rubber and isoprene.

For his work Staudinger received many honours and awards; to mention but a few – he is Dr. Ing. h.c. of the Technische Hochschule Karlsruhe; Dr. rer. nat. h.c. of the University of Mainz; Dr. (C) h.c. of the University of Salamanca; Dr. chem. h.c. of the University of Torino; Dr. sc. techn. h.c. of the Eidgenössische Technische Hochschule Zürich; and Dr. h.c. of the University of Strasbourg. In 1953 he was awarded the Nobel Prize in Chemistry for his discoveries in the field of macromolecular chemistry. In 1933 he was honoured with the Cannizzarro Prize of the Reale Accademia Nazionale dei Lincei in Roma. He is member of the Institut de France, and member and honorary member of many Chemical Societies and the Society of Macromolecular Chemistry in Tokyo.

Hermann Staudinger is married to Magda Woit, who is for many years his co-worker and co-author of numerous publications.

Chemistry 1954

LINUS CARL PAULING

«for his research into the nature of the chemical bond and its application to the elucidation of the structure of complex substances»

Chemistry 1954

Presentation Speech by Professor G. Hägg, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Your Royal Highnesses, Ladies and Gentlemen.

When, in the early nineteenth century, Dalton had produced experimental proofs that matter consists of atoms it was not long before an explanation was sought of the forces that bind the atoms together. Berzelius was of the opinion that this chemical bond was caused by electrostatic attraction between the atoms; according to this belief, a bond was established between two atoms if one of the atoms was positively, and the other negatively charged. In 1819 when Berzelius presented his theory he could apply it almost exclusively to inorganic substances; only few organic substances were known as pure compounds, and the study of these was difficult due to their complicated and often insufficiently known composition. Berzelius, however, contrived to explain, with the help of the new theory, the bond conditions for a great number of inorganic substances, and could in this way contribute in a high degree to a greater clarity in this field.

Even in inorganic chemistry, however, certain difficulties arose. How should one explain, for instance, how two hydrogen atoms unite to become a hydrogen molecule? In order to obtain attraction between atoms, one of the atoms must be positive and the other negative; but why should two atoms of the same kind possess charges with opposite sign? And when the knowledge of organic compounds increased, new difficulties arose. Berzelius, for example, found it necessary to assume that the hydrogen atom was always positive and the chlorine atom always negative. Now it was also found that in organic molecules a hydrogen atom could often be exchanged for a chlorine atom, which should be impossible if one was positive and the other negative.

With increased knowledge, problems that could not be explained by Berzelius' theory became more and more numerous, and the theory became discredited.

After the atomic theory had been accepted, it soon became apparent that another important object in the field of chemistry must be to determine not only the nature of the chemical bond but also how the atoms are arranged

geometrically when they unite to form larger groups, such as molecules. Permit me to quote from a book, remarkable in its day, *Die Chemie der Jetztzeit* written in 1869 by the Swedish chemist Blomstrand:

« It is the important task of the chemist to imitate faithfully in his own way the elaborate constructions which we call chemical compounds, and in the erection of which the atoms have served as building stones, to determine as to number and relative position the points of attack at which one or the other of the atoms attaches itself to the other, in short, to determine the distribution in space of the atoms. »

Blomstrand makes it the aim, therefore, to find the geometrical construction of substances, or what is nowadays called their structure.

At the end of the last century it became obvious that one had to consider several different kinds of chemical bond. Thus, the difficulties of the Berzelius theory were also explained. Berzelius' interpretation was in principle correct as regards a very important type of bond, but he had made the mistake of applying it also to bonds of a different type. After Bohr had introduced his atomic theory one could moreover with its help give a fairly satisfactory explanation of the Berzelius bond. As this bond occurs between electrically charged atoms, so-called ions, this bond type has often been called the ionic bond. The most typical ionic bonds unite the atoms in the crystals of simple salts.

The bond which above all others had prevented a general application of the Berzelius theory is now commonly known as the covalent bond. It occurs commonly when atoms unite to form a molecule and was once characterized by the famous American chemist Gilbert Newton Lewis as « *the* chemical bond ». The bond between the two hydrogen atoms in a hydrogen molecule, which, as was said before, could not be explained by Berzelius' theory, is covalent.

For a long time it was difficult to explain the nature of the covalent bond. Lewis, however, succeeded in 1916 in showing that it is brought about by electrons – generally two – which are shared in common by two neighbouring atoms, thereby uniting them. Eleven years later Heitler and London were able to give a quantum-mechanical explanation of the phenomenon. An exact mathematical treatment of the covalent bond, however, was possible only in the simple case where only one electron unites the two atoms, and when these do not contain additional electrons outside the atomic nuclei. Even for the hydrogen molecule, which contains two electrons, the treatment cannot be absolutely exact, and in still more complicated cases the

mathematical difficulties increase rapidly. It has, therefore, been necessary to use approximate methods, and the results depend to a large extent on the choice of suitable methods and the manner of their application.

Linus Pauling has actively contributed towards the development of these methods, and he has applied them with extreme skill. The results have been such as to be easily usable by chemists. Pauling has also eagerly sought to apply his views to a number of structures which have been experimentally determined during the last decades, both in his own laboratory in Pasadena and elsewhere. It is hardly necessary to mention that we have nowadays great possibilities of reaching Blomstrand's objective of determining the distribution of atoms in space. This is principally done by methods of X-ray crystallography involving an examination of how a crystal influences X-rays in certain respects, and then out of the effect seeking to determine how the atoms are placed in the crystal. Pauling's methods have been very successful and have led to observations which have further advanced the theoretical treatment.

But if the structure of a substance is too complicated it may become impossible to make a more direct determination of the structure with X-rays. In such cases it may be possible, from a knowledge of bond types, atomic distances and bond directions, to predict the structure and then examine whether the prediction is supported by the experiments. Pauling has tried this method in his studies of the structure of proteins with which he has been occupied during recent years. To make a direct determination of the structure of a protein by X-ray methods is out of the question for the present, owing to the enormous number of atoms in the molecule. A molecule of the coloured blood constituent hemoglobin, which is a protein, contains for example more than 8,000 atoms.

In the late nineteen thirties Pauling and his colleagues had already begun to determine with X-rays the structure of amino acids and dipeptides, that is to say, compounds of relatively simple structure containing what may be called fragments of proteins. From this were obtained valuable information about atomic distances and bond directions. These values were supplemented by the determination of the probable limits of variation for distances and directions.

On this basis Pauling deduced some possible structures of the fundamental units in proteins, and the problem was then to examine whether these could explain the X-ray data obtained. It has thus become apparent that one of these structures, the so-called α-helix, probably exists in several proteins.

How far Pauling is right in detail still remains to be proved, but he has surely found an important principle in the structure of proteins. His method is sure to prove most productive in continued studies.

It is hardly necessary to question the practical use of the knowledge of the nature of chemical bonds and of the structure of substances. It is obvious that the properties of a substance must largely depend on the strength with which its atoms are united and the nature of the resulting structure. This does not only apply to the physical properties of the substance, for instance hardness and melting point, but also to its chemical properties, that is to say how it participates in chemical reactions. If we know how certain atoms or groups of atoms are placed in a molecule we can often predict how the molecule should react under given conditions. And as every reaction results in the breaking of some bonds and the formation of others the result will largely depend on the relative strength of the different bonds.

Professor Pauling. Since you began your scientific career more than thirty years ago you have covered a diversity of subjects ranging over wide fields of chemistry, physics, and even medicine. It has been said of you that you have chosen to live « on the frontiers of science » and we chemists are keenly aware of the influence and the stimulative effect of your pioneer work.

Wide though your field of activity may be, you have devoted the greater part of your energy to the study of the nature of the chemical bond and the determination of the structure of molecules and crystals.

It is with great satisfaction, therefore, that the Royal Swedish Academy of Sciences has decided to award to you this year's Nobel Prize for Chemistry for your brilliant achievements in this fundamental field of chemistry.

On behalf of the Academy I wish to extend to you our heartiest congratulations, and now ask you to receive from the hands of His Majesty the King, the Nobel Prize for Chemistry for the year 1954.

Linus Pauling

Modern structural chemistry

Nobel Lecture, December 11, 1954

A century ago the structural theory of organic chemistry was developed. Frankland in 1852 suggested that an atom of an element has a definite capacity for combining with atoms of other elements – a definite valence. Six years later Kekulé and Couper, independently, introduced the idea of valence bonds between atoms, including bonds between two carbon atoms, and suggested that carbon is quadrivalent. In 1861 Butlerov, making use for the first time of the term « chemical structure », stated clearly that the properties of a compound are determined by its molecular structure, and reflect the way in which atoms are bonded to one another in the molecules of the compound. The development of the structure theory of organic chemistry then progressed rapidly, and this theory has been of inestimable value in aiding organic chemists to interpret their experimental results and to plan new experiments.

A most important early addition to organic structure theory was made by the first Nobel Laureate in Chemistry, Van 't Hoff, who in 1874 recognized that the optical activity of carbon compounds can be explained by the postulate that the four-valence bonds of the carbon atom are directed in space toward the corners of a tetrahedron.

The structure theory of inorganic chemistry may be said to have been born only fifty years ago, when Werner, Nobel Laureate in Chemistry in 1913, found that the chemical composition and properties of complex inorganic substances could be explained by assuming that metal atoms often coordinate about themselves a number of atoms different from their valence, usually four atoms at the corners either of a tetrahedron or of a square coplanar with the central atom, or six atoms at the corners of an octahedron. His ideas about the geometry of inorganic complexes were completely verified twenty years later, through the application of the technique of X-ray diffraction.

After the discovery of the electron many efforts were made to develop an electronic theory of the chemical bond. A great contribution was made in 1916 by Gilbert Newton Lewis, who proposed that the chemical bond,

such as the single bond between two carbon atoms or a carbon atom and a hydrogen atom represented by a line in the customary structural formula for ethane, consists of a pair of electrons held jointly by the two atoms that are bonded together. Lewis also suggested that atoms tend to assume the electronic configuration of a noble gas, through the sharing of electrons with other atoms or through electron transfer, and that the eight outermost electrons in an atom with a noble-gas electronic structure are arranged tetrahedrally in pairs about the atom. Applications of the theory and additional contributions were made by many chemists, including Irving Langmuir and Nevil Vincent Sidgwick.

After the discovery of quantum mechanics in 1925 it became evident that the quantum mechanical equations constitute a reliable basis for the theory of molecular structure. It also soon became evident that these equations, such as the Schrödinger wave equation, cannot be solved rigorously for any but the simplest molecules. The development of the theory of molecular structure and the nature of the chemical bond during the past twenty-five years has been in considerable part empirical – based upon the facts of chemistry – but with the interpretation of these facts greatly influenced by quantum mechanical principles and concepts.

The solution of the wave equation for the hydrogen molecule-ion by Ø. Burrau (*Kgl. Danske Videnskab. Selskab, Mat. Fys. Medd.*, 7, (1927) 14) completely clarified the question of the nature of the one-electron bond in this molecule-ion. Two illuminating quantum mechanical discussions of the shared-electron-pair bond in the hydrogen molecule were then simultaneously published, one by Heitler and London (*Z. Physik*, 44 (1927) 455), and the other by E. U. Condon (*Proc. Natl. Acad. Sci. U.S.*, 13 (1927) 466). In the approximate solution of the wave equation for the hydrogen molecule by Heitler and London a wave function is used that requires the two electrons to be separated, each being close to one of the two nuclei. The treatment by Condon permits the electrons to be distributed between the two nuclei independently of one another, each occupying a wave function similar to Burrau's function for the hydrogen molecule-ion. Condon's treatment is the prototype of the molecular-orbital treatment that has been extensively applied in the discussion of aromatic and conjugated molecules, and Heitler and London's treatment is the prototype of the valence-bond method. When the effort is made to refine the two treatments they tend to become identical.

These early applications of quantum mechanics to the problem of the nature of the chemical bond made it evident that in general a covalent bond,

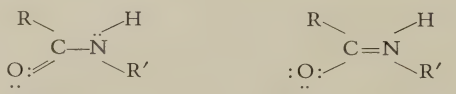
involving the sharing of a pair of electrons between two atoms, can be formed if two electrons are available (their spins must be opposed, in order that the bond be formed), and if each atom has available a stable electronic orbital for occupancy by the electrons.

The equivalence of the four bonds formed by a carbon atom, which had become a part of chemical theory, was not at first easily reconciled with the quantum mechanical description of the carbon atom as having one $2s$ orbital and three $2p$ orbitals in its outer shell. The solution to this difficulty was obtained when it was recognized that as a result of the resonance phenomenon of quantum mechanics a tetrahedral arrangement of the four bonds of the carbon atom is achieved[1]. The carbon atom can be described as having four equivalent tetrahedral bond orbitals, which are hybrids of the s and p orbitals. Further study of this problem led to the discovery of many sets of hybrid bond orbitals, which could be correlated with bond angles, magnetic moments, and other molecular properties[2]. In particular it was found that sp^3, dsp^2, and d^2sp^3 hybrid orbitals correspond respectively to the tetrahedral, square planar, and octahedral configurations of inorganic complexes that had been discovered by Werner. Conclusions as to the utilization of atomic orbitals in bond formation can be drawn from experimental values of magnetic moments. For example, the theory of the dsp^2 square complexes of bipositive nickel, palladium, and platinum requires that these substances be diamagnetic. The square complexes of bipositive palladium and platinum had been recognized by Werner and their structure verified by Dickinson (*J. Am. Chem. Soc.*, 44 (1922) 2404); but the assignment of the square configuration to the complexes of nickel which are diamagnetic had not been made until the development of the new theory.

Further detailed information about the chemical bond resulted from a consideration of the energy of single bonds in relation to the relative electronegativity of the bonded atoms[3]. It was found that the elements can be assigned electronegativity values such as to permit the rough prediction of the heats of formation of compounds to which chemical structures involving only single bonds are conventionally assigned, and that many of the properties of substances can be discussed in a simple way with the use of the electronegativity values of the elements.

The idea that the properties of many organic compounds, especially the aromatic compounds, cannot be simply correlated with a single valence-bond structure, but require the assignment of a somewhat more complex electronic structure, was developed during the period 1923 to 1926 by a

number of chemists, including Lowry, Lapworth, Robinson, and Ingold in England, Lucas in the United States, and Arndt and Eistert in Germany. It was recognized that the properties of aromatic and conjugated molecules can be described by the use of two or more valence-bond structures, as reflected in the names, the theory of mesomerism and the theory of intermediate states, proposed for the new chemical theory. In 1931 Slater, E. Hückel, and others recognized that these theories can be given a quantum mechanical interpretation: an approximate wave function for a molecule of this sort can be set up as the sum of wave functions representing the hypothetical structures corresponding to the individual valence-bond structures. The molecule can then be described as having a structure that is a hybrid of the individual valence-bond structures, or as resonating among these structures, and the theory itself is now usually called the resonance theory of chemical structure. Very many quantitative calculations, approximate solutions of the wave equation, for aromatic and conjugated molecules have been made, with results that are in general in good agreement with experiment. Perhaps more important than the quantitative calculations is the possibility of prediction by simple chemical arguments. For example, the amide group, an important structural feature of proteins, can be described as resonating between two structures, one with the double bond between the carbon atom and the oxygen atom, and the other with the double bond between the carbon atom and the nitrogen atom[4]:



General arguments about the stability of alternative structures indicate that the structure with the double bond between carbon and oxygen should contribute somewhat more to the normal state of the amide group than the other structure; experience with other substances and acquaintance with the results of quantum mechanical calculations suggest the ratio 60%:40% for the respective contributions of these structures. A 40% contribution of the structure with the double bond between the carbon atom and the nitrogen atom would confer upon this bond the property of planarity of the group of six atoms; the resistance to deformation from the planar configuration would be expected to be 40% as great as for a molecule such as ethylene, containing a pure double bond, and it can be calculated that rotation of one end by 3° relative to the other end would introduce a strain energy of 100 cal/mole. The estimate of 40% double-bond character for the C–N bond

is supported by the experimental value of the bond length, 1.32 Å, interpreted with the aid of the empirical relation between double-bond character and interatomic distance[5]. Knowledge of the structure of amides and also of the amino acids, provided by the theory of resonance and verified by extensive careful experimental studies made by R. B. Corey and his coworkers, has been of much value in the determination of the structure of proteins.

In the description of the theory of resonance in chemistry there has been a perhaps unnecessarily strong emphasis on its arbitrary character. It is true, of course, that a description of the benzene molecule can be given, in quantum mechanical language, without any reference to the two Kekulé structures, in which double bonds and single bonds alternate in the ring. An approximate wave function for the benzene molecule may be formulated by adding together two functions, representing the two Kekulé structures, and adding other terms, to make the wave function approximate the true wave function for the molecule more closely, or it may be constructed without explicit introduction of the wave functions representing the two Kekulé structures. It might be possible to develop an alternative simple way of discussing the structure of the amide group, for example, that would have permitted chemists to predict its properties, such as planarity; but in fact no simple way of discussing this group other than the way given above, involving resonance between two valence-bond structures, has been discovered, and it seems likely that the discussion of complex molecules in terms of resonance among two or more valence-bond structures will continue in the future to be useful to chemists, as it has been during the past twenty years.

The convenience and usefulness of the concept of resonance in the discussion of chemical problems are so great as to make the disadvantage of the element of arbitrariness of little significance. Also, it must not be forgotten that the element of arbitrariness occurs in essentially the same way in the simple structure theory of organic chemistry as in the theory of resonance – there is the same use of idealized, hypothetical structural elements. In the resonance discussion of the benzene molecule the two Kekulé structures have to be described as hypothetical: it is not possible to synthesize molecules with one or the other of the two Kekulé structures. In the same way, however, the concept of the carbon–carbon single bond is an idealization. The benzene molecule has its own structure, which cannot be exactly composed of structural elements from other molecules. The propane molecule also has its own structure, which cannot be composed of structural elements from other

molecules – it is not possible to isolate a portion of the propane molecule, involving parts of two carbon atoms and perhaps two electrons in between them, and say that this portion of the propane molecule is the carbon–carbon single bond, identical with a portion of the ethane molecule. The description of the propane molecule as involving carbon–carbon single bonds and carbon–hydrogen single bonds is arbitrary; the concepts themselves are idealizations, in the same way as the concept of the Kekulé structures that are described as contributing to the normal state of the benzene molecule. Chemists have found that the simple structure theory of organic chemistry and also the resonance theory are valuable, despite their use of idealizations and their arbitrary character.

Other extensions of the theory of the chemical bond made in recent years involve the concept of fractional bonds. Twenty-five years ago it was discovered that a simple theory of complex crystals with largely ionic structures, such as the silicate minerals, can be developed on the basis of the assumption that each cation or metal atom divides its charge or valence equally among the anions that are coordinated about it[6]. For example, in a crystal of topaz, $Al_2SiO_4F_2$, each silicon atom is surrounded by a tetrahedron of four oxygen atoms, and each aluminum atom is surrounded by an octahedron of four oxygen atoms and two fluorine atoms. The valence of silicon, 4, is assumed to be divided among four bonds, which then have the bond number 1 – they are single bonds. The valence of aluminum, 3, is divided among six bonds, each of which is a half bond. A stable structure results when the atoms are arranged in such a way that each anion, oxygen or fluorine, forms bonds equal to its valence. In topaz each oxygen atom forms one single bond with silicon and two half bonds with aluminum, whereas each fluorine atom forms only two half bonds with aluminum. The distribution of the valences hence then corresponds to the bivalence of oxygen and the univalence of fluorine. It was pointed out by W. L. Bragg that if the metal atoms are idealized as cations (Si^{++++} and Al^{+++}) and the oxygen and fluorine atoms as anions (O^{--} and F^{-}), this distribution corresponds to having the shortest possible lines of force between the cations and the anions – the lines of force need to reach only from a cation to an immediately adjacent anion, which forms part of its coordination polyhedron. Occasionally ionic crystals are found in which there are small deviations from this requirement, but only rarely are the deviations larger than one quarter of a valence unit.

Another application of the concept of fractional valence bonds has been made in the field of metals and alloys. In the usual quantum mechanical

discussion of metals, initiated by W. Pauli (*Z. Physik*, 41 (1927) 81) and Sommerfeld (*Naturwiss.*, 15 (1927) 825), the assumption was made that only a small number of electrons contribute significantly to the binding together of the metal atoms. For example, it was customary to assume that only one electron, occupying a 4*s* orbital, is significantly involved in the copper–copper bonds in the metal copper. Sixteen years ago an analysis of the magnetic properties of the transition metals was made that indicated that the number of bonding electrons in the transition metals is much larger, of the order of magnitude of six[7]. Iron, for example, can be described as having six valence electrons, which occupy hybrid d^3sp^2 orbitals. The six bonds, corresponding to these six valence electrons, resonate among the fourteen positions connecting an iron atom with its fourteen nearest neighbors. The bonds to the eight nearest neighbors have bond number approximately $\frac{5}{8}$, and those to the six slightly more distant neighbors have bond number $\frac{1}{6}$. In gamma iron, where each atom is surrounded by twelve equally distant neighbors, the bonds are half bonds. The concept that the structure of metals and intermetallic compounds can be described in terms of valence bonds that resonate among alternative positions, aided by an extra orbital on most or all of the atoms (the metallic orbital), has been found of value in the discussion of the properties of these substances[8]. The resonating-bond theory of metals is supported especially strongly by the consideration of interatomic distances in metals and intermetallic compounds[9].

The iron atom has eight electrons outside of the argon shell of eighteen. Six of these electrons are assumed, in the resonating-valence-bond theory, to be valence electrons, and the remaining two are atomic electrons, occupying 3*d* orbitals, and contributing two Bohr magnetons to the magnetic moment of the atom. A theory of the ferromagnetism of iron has recently been developed[10], in which, as suggested by Zener (*Phys. Rev.*, 81 (1951) 440), the interaction producing the Weiss field in the ferromagnetic metal is an interaction of the spin moments of the atomic electrons and uncoupled spins of some of the valence electrons. It has been found possible to use spectroscopic energy values to predict the number of uncoupled valence electrons, and hence the saturation magnetic moment for iron: the calculation leads to 0.26 uncoupled valence electrons per atom, and saturation magnetic moment 2.26 Bohr magnetons, which might be subject to correction by two or three percent because of the contribution of orbital moment. The experimental value is 2.22. A calculated value of the Curie temperature in rough agreement with experiment is also obtained.

The valence theory of metals and intermetallic compounds is still in a rather unsatisfactory state. It is not yet possible to make predictions about the composition and properties of intermetallic compounds with even a small fraction of the assurance with which they can be made about organic compounds and ordinary inorganic compounds. We may, however, hope that there will be significant progress in the attack on this problem during the next few years.

Let us now return to the subject of the structural chemistry of organic substances, especially the complex substances that occur in living organisms, such as proteins. Recent work in this field[11] has shown the value of the use of structural arguments that go beyond those of the classical structure theory of organic chemistry. The interatomic distances and bond angles in the polypeptide chains of proteins are precisely known, the bond distances to within about 0.02 Å and the bond angles to within about 2°. It is known that the amide groups must retain their planarity; the atoms are expected not to deviate from the planar configuration by more than perhaps 0.05 Å. There is rotational freedom about the single bonds connecting the alpha carbon atom with the adjacent amide carbon and nitrogen atoms, but there are restrictions on the configurations of the polypeptide chain that can be achieved by rotations about these bonds: atoms of different parts of the chain must not approach one another so closely as to introduce large steric repulsion, and in general the N–H and O atoms of different amide groups must be so located relative to one another as to permit the formation of hydrogen bonds, with N–H$\cdots$O distance equal to 2.79 ± 0.10 Å and with the oxygen atom not far from the N–H axis. These requirements are stringent ones. Their application to a proposed hydrogen-bonded structure of a polypeptide chain cannot in general be made by the simple method of drawing a structural formula; instead, extensive numerical calculations must be carried out, or a model must be constructed. For the more complex structures, such as those that are now under consideration for the polypeptide chains of collagen and gelatin, the analytical treatment is so complex as to resist successful execution, and only the model method can be used. In order that the principles of modern structural chemistry may be applied with the power that their reliability justifies, molecular models must be constructed with great accuracy. For example, molecular models on the scale 2.5 cm = 1 Å have to be made with a precision better than 0.01 cm.

We may, I believe, anticipate that the chemist of the future who is interested in the structure of proteins, nucleic acids, polysaccharides, and other

complex substances with high molecular weight will come to rely upon a new structural chemistry, involving precise geometrical relationships among the atoms in the molecules and the rigorous application of the new structural principles, and that great progress will be made, through this technique, in the attack, by chemical methods, on the problems of biology and medicine.

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Biography

Linus Carl Pauling was born in Portland, Oregon, on 28th February, 1901, the son of a druggist, Herman Henry William Pauling, who, though born in Missouri, was of German descent, and his wife, Lucy Isabelle Darling, born in Oregon of English-Scottish ancestry.

Linus attended the public elementary and high schools in the town of Condon and the city of Portland, Oregon, and entered the Oregon State College in 1917, receiving the degree of B.Sc. in chemical engineering in 1922. During the years 1919–1920 he served as a full-time teacher of quantitative analysis in the State College, after which he was appointed a Teaching Fellow in Chemistry in the California Institute of Technology and was a graduate student there from 1922 to 1925, working under Professor Roscoe G. Dickinson and Richard C. Tolman. In 1925 he was awarded the Ph.D. (*summa cum laude*) in chemistry, with minors in physics and mathematics.

Since 1919 his interest lay in the field of molecular structure and the nature of the chemical bond, inspired by papers by Irving Langmuir on the application of the Lewis theory of the sharing of pairs of electrons between atoms to many substances. In 1921 he suggested, and attempted to carry out, an experiment on the orientation of iron atoms by a magnetic field, through the electrolytic deposition of a layer of iron in a strong magnetic field and the determination of the orientation of the iron crystallites by polishing and etching the deposit, and microscopic examination of the etch figures. With Professor Dickinson, he began in 1922 the experimental determination of the structures of some crystals, and also started theoretical work on the nature of the chemical bond.

Since his appointment to the Staff of California Institute of Technology, Professor Pauling was elected Research Associate in 1925; National Research Fellow in Chemistry, 1925–1926; Fellow of the John Simon Guggenheim Memorial Foundation, 1926–1927 (through this last he worked in European Universities with Sommerfeld, Schrödinger, and Bohr); Assistant Professor of Chemistry, 1927–1929; Associate Professor, 1929–1931; Professor, 1931, when he was the first recipient of the American Chemical Society Award in

Pure Chemistry – the Langmuir Prize – and Chairman of the Division of Chemistry and Chemical Engineering, and Director of the Gates and Crellin Laboratories of Chemistry, 1936–1958. In 1963, he was awarded the Nobel Peace Prize.

Pauling is a member of numerous professional societies in the U.S.A. as well as in many European countries, India, Japan and Chile. Awards, medals, and honorary degrees were showered upon him in America and Europe, and in addition he was elected Rationalist of the Year for 1960 and Humanist of the Year for 1961. Several books have come from his pen, ranging from his most famous one *The Nature of the Chemical Bond, and the Structure of Molecules and Crystals* (1939, 1949, 1960) via *General Chemistry* (1947, 1953), which was translated into nine languages, to *No More War!* (1958, 1959, 1962).

The subjects of the papers he published reflect his great scientific versatility: about 350 publications in the fields of experimental determination of the structure of crystals by the diffraction of X-rays and the interpretation of these structures in terms of the radii and other properties of atoms; the application of quantum mechanics to physical and chemical problems, including dielectric constants, X-ray doublets, momentum distribution of electrons in atoms, rotational motion of molecules in crystals, Van der Waals forces, etc.; the structure of metals and intermetallic compounds, the theory of ferromagnetism; the nature of the chemical bond, including the resonance phenomenon in chemistry; the experimental determination of the structure of gas molecules by the diffraction of electrons; the structure of proteins; the structure of antibodies and the nature of serological reactions; the structure and properties of hemoglobin and related substances; abnormal hemoglobin molecules in relation to the hereditary hemolytic anemias; the molecular theory of general anesthesia; an instrument for determining the partial pressure of oxygen in a gas; and other subjects.

Pauling married Ava Helen Miller of Beaver Creek, Oregon, in 1923. She is of English-Scottish and German descent. They have four children, Linus (Carl) Jr. (1925), Peter Jeffress (1931), Linda Helen (1932) and Edward Crellin (1937), and thirteen grandchildren.

Chemistry 1955

VINCENT DU VIGNEAUD

«for his work on biochemically important sulphur compounds, especially for the first synthesis of a polypeptide hormone»

Chemistry 1955

Presentation Speech by Professor A. Fredga, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Your Royal Highnesses, Ladies and Gentlemen.

The word sulphur may give rise to rather disparate sensations; to most people it has not a very pleasing ring. Also many chemists are inclined to keep their distance; they know that organic sulphur compounds may infect the atmosphere around wood pulp mills and that they form part of the chemical weapon of the common skunk. In spite of that, the element sulphur is of vital importance, and it has many functions in the living organism.

Professor du Vigneaud once defined true exploratory research as «the working out of a winding trail into the unknown». He himself has followed the trail of sulphur in metabolism.

The story starts in the nineteen-twenties with insulin, a substance whose importance is now well-known. As to its chemical nature, it is a protein with a remarkably high percentage of sulphur. The proteins are built up from so-called amino acids, and their sulphur is due to the presence of one or two sulphur-containing amino acids: cystine and methionine. Considering the special properties of insulin, one could perhaps expect to find its sulphur in quite a new combination, but it turned out that it was present in cystine. The result may seem to be of little significance, but the trail pointed in a definite direction; the sulphur-containing amino acids must be more closely investigated.

It would lead too far to give an account of Professor du Vigneaud's numerous works on cystine, methionine, and related compounds. They led, *inter alia*, to the discovery of the biological importance of the methyl group and of its migrations in the organism. I must also leave aside the distinguished works on biotin, also known as vitamin H, and coenzyme R, and the important contributions to the chemistry of penicillin.

Underneath the brain, there is a small, well-protected gland, the pituitary gland. In man it is about as big as a bean. There are secreted several hormones, that is, substances which regulate important physiological functions. In spite of its small size, the pituitary gland is made up of several distinct parts with different functions. We are interested here in the posterior lobe, which

contains two substances called oxytocin and vasopressin. The former stimulates the contractions of the uterus and also the lactation, the latter raises the blood pressure and regulates the function of the kidneys. As early as in 1933, when rather impure preparations from the posterior lobe were used in experiments, du Vigneaud found a high percentage of sulphur, which seemed to be correlated to the physiological activity.

Using the experimental methods, which the development of science has put at his disposal and making the best of his own intimate knowledge of the organic chemistry of sulphur, du Vigneaud has step by step forced his way. Both hormones were isolated in a state of purity, and it was found that they are built up from amino acids in the same way as proteins, but with a far lower molecular weight. Such compounds are, as distinguished from real proteins, called polypeptides. The nature of the amino acids and their positions in the molecule could be determined. The sulphur is present in cystine. The two hormones have a very similar structure; both contain eight amino acids, connected to a chain, which at one point is closed to a ring. The molecule has some resemblance to a figure six or nine, where the loop contains five amino acids and the «tail» three. Two sulphur atoms, linked to each other, form a part of the ring.

The design of the molecule was thus known. It remained to build it up by synthesis and check the correctness of the design. That was perhaps the most difficult part of the work. The interest was first concentrated on the synthesis of oxytocin. Step by step the amino-acid chain was built up with the two sulphur atoms in the proper positions, one at the end of the chain and the other near the middle. At last the ring was closed by formation of a bond between the sulphur atoms. Now followed the most thrilling moment, the testing of the chemical properties and the physiological activity; perhaps there had been some mistake after all. It turned out, however, that the synthetic polypeptide was identical with the natural product.

A biologically important substance of complicated structure had been investigated and synthesized with eminent skill. However, more important still are the far-reaching consequences of this achievement. It is the first synthesis of a polypeptide hormone. It also shows, that such substances with very important physiological properties may be built up from well-known amino acids according to well-known principles. The eight amino acids must, however, be combined in a special sequence; it may not be out of place to draw attention to the principle of the combination lock. The result may also be of importance for revealing the secrets of the real proteins.

Professor du Vigneaud. Some years ago you gave a survey of your most important works and you called it: « A trail of research in sulphur chemistry and metabolism ». At an early stage you found that the element sulphur has a special fascination, and you started out to follow its winding trail into the unknown. It has led you to many important discoveries and recently to the first synthesis of a polypeptide hormone. We are sure it will lead you further still.

In the structure of these hormones there is nothing mysterious, nothing thrilling. They are built up like proteins, and the building-stones, the eight amino acids, are those found in common proteins. The conspicuous properties must be due to the sequence, the arrangement. This is a very important step on the way, leading to the understanding of the mode of action of such hormones.

In our days, there is often a tendency to draw a borderline between organic and biological chemistry. If you have heard about this borderline, you have never cared for it. Your work is a triumph for organic chemistry, but the most important consequences fall within the regions of biochemistry and physiology. In recognition of your services to Natural Science, the Royal Academy has decided to bestow upon you the Nobel Prize for Chemistry for your work on sulphur compounds of biochemical importance, and especially for the first synthesis of a polypeptide hormone. To me has been granted the privilege of conveying to you the warmest congratulations of the Academy and of inviting you to receive your prize from the hands of His Majesty the King.

Vincent du Vigneaud

A trail of sulfur research: from insulin to oxytocin

Nobel Lecture, December 12, 1955

It is a pleasure to have the opportunity of discussing with this Academy some of the researches that I have been interested in during the past thirty years. I am deeply grateful to the Academy for the recognition, as expressed in the terms of the citation, of our work on « biochemically important sulfur compounds ». You have also noted in the citation « the first synthesis of a polypeptide hormone ». This hormone, oxytocin, the principal uterine-contracting and milk-ejecting hormone of the posterior pituitary gland, is a sulfur-containing compound, and its synthesis was the culmination of many experiences along a trail of research stemming from my original interest in sulfur and in insulin.

It would seem proper, therefore, with the citation in mind, that I should discuss with you a « trail of sulfur research ». I shall try to unravel some of the stepwise evolvement of our researches from insulin to oxytocin. I shall also try to bring out the background of the research findings and the thinking behind the researches.

I have had the pleasure of following this trail of research in the company of a group of graduate students and postdoctoral associates, without whose loyal and effective collaboration this trail I am going to present could not have been worked out.

I have also been fortunate in the kind of support that I have received from various foundations, such as the Rockefeller Foundation; from industrial firms, such as Parke, Davis and Company, Eli Lilly and Company, and the Lederle Laboratories Division of the American Cyanamid Company; from a governmental agency, namely the National Institutes of Health; and from the universities where I have had the pleasure of working. All of this support was unrestricted, allowing us to explore whatever trail we wished.

This trail becomes apparent only in retrospect. Obviously, I did not start out to study sulfur as my life's work. And yet, as I look back over the trail of many years, I encounter the fact that this thread of sulfur has been the thread of continuity running through practically all of my research endeavors.

I find it intriguing to contemplate how one starts out on a trail of exploration in the laboratory, not knowing where one is eventually going, starting out, to be sure, with some immediate objective in mind, but also having a vague sense of something beyond the immediate objective towards which one is striving. The thrill of this kind of research, albeit at a sublimated level, is analogous, I am sure, to the thrill that explorers like the Vikings of old experienced in breaking through the confines of the known world.

As one looks back on a trail of research, the continuity is sometimes greater than one may have imagined at the time. As I look back, it seems almost inevitable that I should have proceeded from the pancreatic hormone, insulin, to the posterior pituitary hormone, oxytocin, via sulfur. Again, in looking back, I now realize that there were times when we stopped to work out more fully the chemistry of other sulfur-containing compounds, such as cystine, homocystine, and methionine, and to study their metabolic significance in transsulfuration and transmethylation. These researches diverted us into interesting by-paths. However, strangely enough, as I shall try to bring out, many of these experiences, particularly the cystine peptide work and the knowledge of certain reactions involving sodium and liquid ammonia which we gained along the route, were vital when we came to study the posterior pituitary hormones.

Now where did the sulfur trail start?

I think it started at the University of Illinois, where my first teacher in biochemistry was the late Professor H. B. Lewis, who was extremely enthusiastic about sulfur. It was his enthusiasm that undoubtedly aroused my interest in the biochemistry of sulfur compounds.

In my work in organic chemistry at that same university with Professor C. S. Marvel, I developed a strong interest in the relationship of organic chemical structure to biological activity. The continuing interest in this relationship has also influenced greatly the direction of this trail of research. The work I did with Professor Marvel was concerned with the synthesis of compounds which we hoped would have a local anaesthetic effect accompanied by an epinephrine-like effect. As I look back over the trail, I find this theme – the relation between biological activity and organic chemical structure – recurring again and again.

My interest in insulin was initiated through a lecture given by Professor W. C. Rose, who succeeded Lewis as Professor of Biochemistry at Illinois. On his return from a meeting in Toronto in 1923, he gave an account of the exciting discovery of insulin by Banting and Best. I will recall the thrill of

listening to Professor Rose and my curiosity as to the chemical nature of a compound that could bring about the miracles he described. Little did I know at that time that insulin would eventually turn out to be a sulfur compound.

Some two years later, I received an invitation from Professor J. R. Murlin at the University of Rochester, Medical School, to come and work on the chemistry of insulin in his department, a department devoted mainly to endocrinology and metabolism. The chance to work on the chemistry of insulin transcended all other interests for me, and I accepted Professor Murlin's invitation.

While there, I became intrigued with the fact that all of our preparations contained sulfur, and most of my efforts over the next two years were devoted to studying the sulfur of these insulin preparations. From these studies I came to the conclusion that the sulfur was present in the form of the disulfide linkage and that insulin was most likely a derivative of the amino acid cystine, and the suggestion was made that the cystine in insulin was linked to the rest of the molecule by peptide linkages[1].

The following year, while working in Professor Abel's laboratory at Johns Hopkins University, I took up the isolation of cystine from crystalline insulin, because the conclusive proof of the presence of cystine in insulin had to rest on the isolation of the cystine in pure form. This isolation was eventually accomplished[2]. As I continued work at Hopkins on insulin in collaboration with Jensen and Wintersteiner, we could find nothing but ordinary amino acids and ammonia in acid hydrolysates of insulin[3,4].

The presence of cystine in insulin naturally brought many questions to mind. One of the first questions that occurred to me was whether various combinations of cystine with other amino acids in peptide linkage might affect the lowering of blood sugar. It was then realized that as yet no peptide of cystine linked through the carboxyl group of cystine had been prepared. No method was available at the time for the synthesis of this type of peptide. Therefore, while the researches on insulin were continued over the next several years, parallel studies were carried out with several graduate students on the synthesis of peptides of cystine.

Although the peptides we eventually made did not have hypoglycemic activity, the work thereon gave us valuable experience in the synthesis of cystine peptides and also led to a synthesis of the biologically important compound, glutathione, a tripeptide of glutamic acid, cysteine, and glycine. This work on cystine peptides led to the development by us of several reac-

tions involving sodium and liquid ammonia, which, almost twenty years later, played a vital role in our synthesis of oxytocin, which will be discussed subsequently. One of these reactions was the removal of a carbobenzoxy group by sodium in liquid ammonia, and another was the utilization of a benzyl group to cover the sulfur of cysteine during certain synthetic steps and its final removal by means of sodium in liquid ammonia.

As is well known, Bergmann and Zervas introduced in 1932 the now classical method of protecting an amino group during the course of peptide synthesis with what they called a carbobenzoxy group[5]. Their procedure led to the carbobenzoxy derivative of the peptide, and the carbobenzoxy group was removed by catalytic reduction with hydrogen. However, their procedure did not lend itself to the preparation of cysteine or cystine peptides.

Two years after the appearance of the Bergmann–Zervas method, it occurred to me that the carbobenzoxy group might possibly be cleaved from the amino group of cystine by reduction with sodium in liquid ammonia. If so, a very convenient method for the synthesis of cystine peptides might result. In work with Audrieth and Loring[6], we had already prepared cysteine from cystine by this method of reduction.

The reduction of dicarbobenzoxycystine was therefore attempted in work with Sifferd[7]. The compound was dissolved in liquid ammonia and sodium was added until a permanent blue color was obtained. After evaporation of the ammonia and subsequent oxidation of the cysteine by aeration of the slightly alkaline solution, cystine was obtained in almost quantitative yield.

In our earlier work on the preparation of cysteine, it had occurred to us that it might be possible to benzylate the sulfhydryl group of cysteine by adding benzyl chloride to the liquid ammonia solution of the sodium salt of cysteine produced by the reduction of cystine with metallic sodium. An excellent yield of *S*-benzylcysteine was obtained. Although the latter reaction was carried out in 1930 with Loring and Audrieth, it was not until sometime later that the possibility of cleaving a benzyl thio ether by this same means occurred to us in our work with Sifferd. *S*-Benzylcysteine was cleaved to cysteine in liquid ammonia with metallic sodium, cystine being isolated after oxidation[7].

This same reductive procedure was also applied to the preparation of cystinylbisglycine from dicarbobenzoxycystinylbisglycine and of cysteinylglycine from *S*-benzylcysteinylglycine[8].

The effectiveness of these reactions impressed us with their potentialities as possible key reactions for a synthesis of glutathione, the structure of which

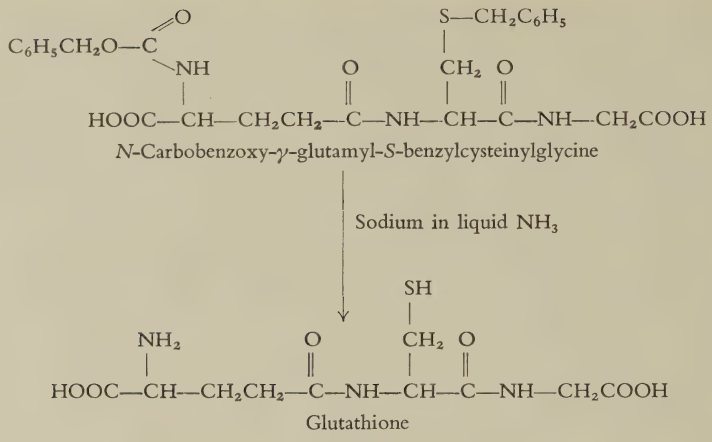

Fig. 1. A synthesis of glutathione.

was believed, through the work of Hopkins[9] and of Kendall, Mason, and McKenzie[10] to be γ-L-glutamyl-L-cysteinylglycine. If we could synthesize *N*-carbobenzoxy-γ-glutamyl-*S*-benzylcysteinylglycine, we felt that its reduction with sodium in liquid ammonia should yield glutathione.

This approach to glutathione would have a particular advantage in that the sulfhydryl group would be covered by a benzyl radical up to the final step, and thus, during the course of the various reactions that might be employed up to this point, the likelihood of partial oxidation of the sulfhydryl group and its attendant difficulties would be eliminated.

In work with Miller[11], we were able to obtain this desired intermediate by the coupling of suitable derivatives of the three amino acids involved. As shown in Fig. 1, reduction of this intermediate with sodium in liquid ammonia gave glutathione, which, upon isolation in crystalline form, was shown to be identical with the natural product. Our synthesis followed shortly after the first synthesis of glutathione by Harington and Mead by a somewhat different approach[12].

While this synthetic work was going on, we continued our work on insulin, along mainly two lines. One was concerned with whether the cystine content accounted for all of the sulfur, and the second with the behavior of insulin upon reduction of the disulfide groups. After some seven years of work, we finally were able to account for the sulfur of insulin entirely on the basis of cystine[13], and we could obtain no evidence for any sulfur compound other than cystine in insulin[14,15].

The study of the reduction of insulin led directly to our work on the

posterior pituitary hormones. With such a gentle reducing agent as cysteine or glutathione acting at room temperature and at a neutral pH, insulin became inactivated, reduction of the disulfide linkages being undoubtedly the cause of the inactivation[16–18]. Reoxidation did not restore activity.

This work on insulin aroused our interest in other protein or protein-like hormones. We turned to the examination of oxytocin, the uterine-contracting hormone, and vasopressin, the blood-pressure-raising hormone, of the posterior pituitary gland. There were some indications in the literature that these hormones might be polypeptide-like substances of lower molecular weight than insulin. Furthermore, there was evidence that partially purified preparations of these hormones contained sulfur, but the nature of the sulfur was unknown. We thought it would be interesting to investigate these hormones in comparison with insulin, and in 1932 we made some preliminary explorations on these hormones. In this discussion I will confine my attention mainly to oxytocin with only occasional reference to vasopressin.

Kamm and Grote of Parke, Davis and Company kindly placed at our disposal some of their partially purified oxytocin, and we were able to show that, upon hydrolysis, the samples contained approximately 9 per cent cystine[19]. Of course we couldn't tell at that time whether the cystine was present in the hormone or in the impurities. Nevertheless, in work with Sealock[20], we decided to treat the partially purified oxytocin with cysteine and find out whether this hormone lost its activity like insulin. Much to our surprise, the oxytocic activity remained. Oxidation, by aeration of an aqueous solution until the sulfhydryl test was negative, did not cause loss of activity. The question then occurred, had we really reduced the hormone by the cysteine treatment? It appeared possible to us that if the hormone were a disulfide and had been reduced, then treatment with benzyl chloride might cover the sulfhydryl group with a benzyl radical and inactivation might take place. When the reduced oxytocin preparation was treated with benzyl chloride, inactivation did result. On the other hand, treatment of the non-reduced material with benzyl chloride did not cause inactivation. These results made us fairly certain that the oxytocic principle contained sulfur in the form of a disulfide linkage[20].

We also investigated the behavior of the vasopressin preparation upon cysteine treatment and found it quite parallel to that of the oxytocin preparation. This aroused in us the desire to see what the pure compounds themselves would be like. What manner of compounds were they? Were they, like insulin, also simply made up of amino acids and ammonia? Since

we had reason to believe that they were smaller molecules than insulin, it seemed to me that they might lend themselves to an organic chemical approach. If we could isolate them, we thought we might be able to work out their structure and perhaps synthesize them.

The purification was a slow process, as the amount of active principles in the gland is extremely small, they are unstable, and the bioassays involved are very time-consuming. Hundreds of thousands of hog and beef glands were used during the course of the investigations. Up to the time of World War II, we made considerable progress in collaboration with Sealock, Irving, Dyer, and Cohn on the purification of the principles, mainly through electrophoretic techniques, and learned much about the behavior of the hormones[21–26].

We laid aside the problem during the war period for certain assignments, particularly on penicillin, but thereafter the isolation of oxytocin was undertaken in collaboration with Livermore[27]. Since the countercurrent distribution technique developed by Craig[28] for the purification of organic compounds had played a helpful role in our isolation of synthetic penicillin, we naturally thought of using countercurrent distribution on partially purified oxytocin fractions, prepared by the method of Kamm and co-workers[29]. The source material for preparation of the oxytocin fractions was a commercial extract provided by Dr. Kamm of Parke, Davis and Company. The countercurrent distribution between 0.05 per cent acetic acid and secondary butyl alcohol proved to be highly effective. We obtained a fraction that appeared to behave like a pure compound by this criterion, and through application of the elegant starch-column chromatographic method of Moore and Stein[30] we were able, with Pierce[31], to show that an acid hydrolysate of oxytocin consisted of eight amino acids and ammonia.

It was then of importance to determine whether, starting from the glands themselves, material of the same potency and properties would be obtained. Therefore the oxytocin was isolated from lyophilized posterior lobes of beef pituitary glands[32]. A preparation was obtained which had approximately the same distribution curve and the same potency as the preparation obtained from the concentrate. The two preparations likewise showed the same amino acid composition. The chromatogram of the amino acids is shown in Fig. 2. The amino acids were present in a molar ratio to each other of 1:1, and the molar ratio of ammonia to any one amino acid was 3:1. Molecular weight determinations indicated a molecular weight in the neighborhood of 1,000.

The sulfur content of oxytocin could be entirely accounted for by cystine.

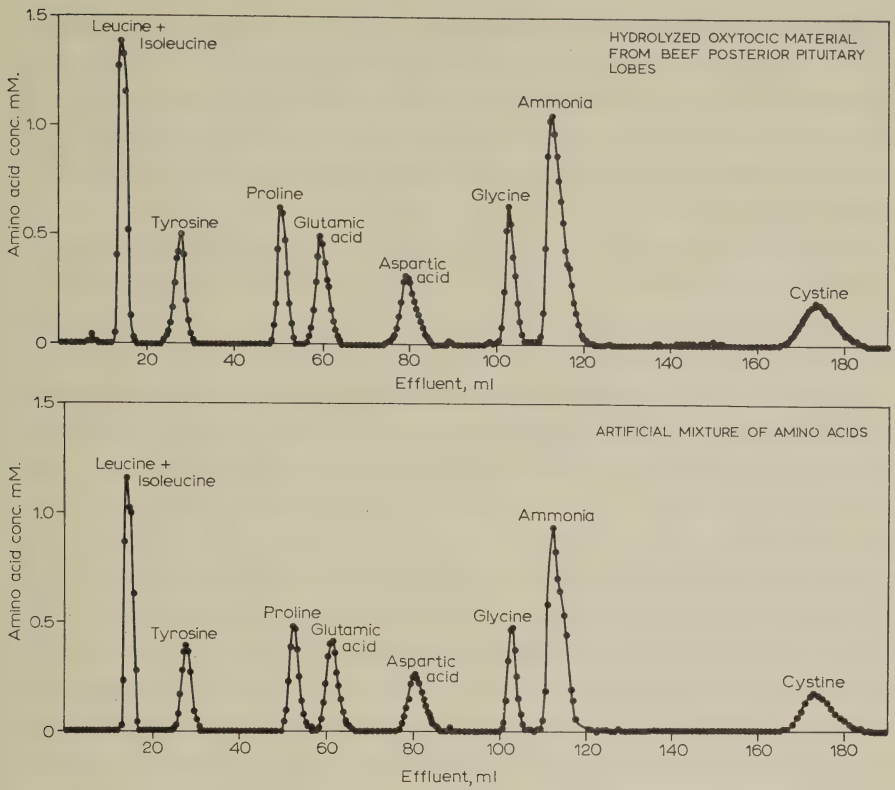

Fig. 2. Separation of amino acids from a hydrolysate of oxytocin (*upper chromatogram*) and from an artificial mixture of amino acids simulating the composition of oxytocin (*lower chromatogram*). Solvents, 1:2:1 *n*-butyl alcohol – *n*-propyl alcohol – 0.1 *N* HCl followed by 2:1 *n*-propyl alcohol – 0.5 *N* HCl.

Knowing the cystine content of the purified hormone, it becomes evident, from the sulfur content of all of the pre-war preparations including those of Kamm and co-workers and our preparations, that none of these preparations could have been more than 50 per cent pure, regardless of the unitage ascribed to them on an arbitrary basis by reference to a standard powder. No greater purity than 50 percent could be attributed to these early preparations, even if the sulfur of the preparations were due entirely to the presence of oxytocin, which is somewhat unlikely since sulfur-containing impurities may well have been present.

Further countercurrent distribution of the purified oxytocin involving 1,000 transfers resulted in no change in composition, and this work[33] culminated in 1952 in the isolation of a crystalline flavianate of oxytocin

with Pierce, the first crystalline derivative of this hormone to be isolated.

It is of interest that an oxytocic fraction was also obtained from hog posterior pituitary glands which had a distribution curve approximately the same as that from the beef glands[33]. In addition, the oxytocin obtained from the hog pituitary had the same amino acid composition and potency as that obtained from beef.

During the course of these studies on the oxytocic hormone, the pressor hormone, vasopressin, was also isolated from beef glands and shown to contain six of the same amino acids as oxytocin. In place of the leucine and isoleucine in oxytocin, vasopressin contained phenylalanine and arginine.

With the isolation of what appeared to be the pure hormones and the establishment of their composition, we were for the first time in a position, on a chemical basis, to be quite certain that the oxytocin was free of vasopressin, and therefore it was possible to ascertain the biological effects of oxytocin itself.

Before going into this, it might be well to mention a few of the biological activities that have been attributed to the posterior pituitary gland. I would recall to you that it was just sixty years ago that the first biological effect of the pituitary gland was discovered by Oliver and Schäfer[34]. They found that extracts of the pituitary when injected into mammals raised their blood pressure – the pressor effect. Howell showed a few years later that this activity resided in the posterior lobe[35]. Since that time, other biological activities of posterior pituitary extracts were noted, particularly the uterine-contracting, or oxytocic, effect by Dale[36] in 1906; the milk-ejecting effect by Ott and Scott[37] in 1910; the blood-pressure-lowering effect in birds, the so-called avian depressor effect, by Paton and Watson[38] in 1912; and the inhibition of urine excretion in man, the antidiuretic effect, by Von den Velden[39] in 1913.

As to the biological effects of the purified oxytocin, it was assayed for avian depressor effect against a standard powder according to the method of Coon as described in the U.S. Pharmacopoeia[40] and found to possess this activity[33] to the extent of 450 to 500 units per mg. In addition to the avian depressor effect, the oxytocin was found to have the same potency, relative to the standard powder, in bringing about contractions of the isolated rat uterus – the uterine-contracting activity. The oxytocin also showed the same potency (450 to 500 units per mg, relative to the standard powder) in bringing about the ejection of milk. This milk-ejecting activity of oxytocin was demonstrated by tests of our purified material in sows by Whittlestone[41], in rabbits by Cross and Van Dyke[42], and in recently parturient women, the

latter testing having been carried out in a collaborative study with Douglas, Nickerson, and Bonsnes of our Department of Obstetrics and Gynecology[43].

We thought at first that oxytocin was devoid of pressor and antidiuretic activity. However, we placed at Van Dyke's disposal samples of our purified oxytocin which he and his colleagues assayed by refined techniques. They found 7 units of pressor and 3 units of antidiuretic activity per mg. These activities have been confirmed qualitatively and quantitatively with our synthetic oxytocin, so there is no longer any question that they are inherent properties of the oxytocin molecule[44]. It might be mentioned that vasopressin, in addition to its pressor and antidiuretic effects (500 to 600 units per mg, relative to the standard powder), also possesses avian depressor, uterine-contracting, and milk-ejecting activity, but the potency of vasopressin with respect to the latter three activities is only a fraction of the potency of oxytocin.

With the purified oxytocin at hand and its composition established, we then turned to the problem of how the component amino acids were linked. Of course there were many structures that could be written involving the eight amino acids and ammonia. The greatest difficulty in the degradative work was the scarcity of material. To obtain enough purified hormones was truly a prodigious task, as has already been mentioned. The various degradative steps were perforce carried out on milligrams of material, and in most instances, the methods had to be adapted to this scale.

Since I am attempting in this presentation to focus attention on the synthesis of oxytocin in relation to this trail of sulfur research, I shall not present the studies involving a variety of procedures in our gradual elucidation of the structure of the hormone. These researches over the course of several years with Pierce, Mueller, Turner, Davoll, Taylor, and Kunkel[45–50], and the final decisive experiments with Ressler and Trippett[51,52] on the cleavage of performic acid-oxidized oxytocin with bromine water and on the partial hydrolysis and identification of peptide fragments, brought us to a clear-cut concept[52,53] of the structure of oxytocin, a new type of cyclic polypeptide amide shown in Fig. 3.

Although this structure was the only one that we could arrive at through the rationalization of our data, we felt that synthetic proof of this structure was mandatory because of certain assumptions involved in postulating it.

It is of considerable interest that Tuppy, on the basis of data we had published along with some data of his own, arrived at the same structure independently[54]. Tuppy's proposal was based on the data from our labora-

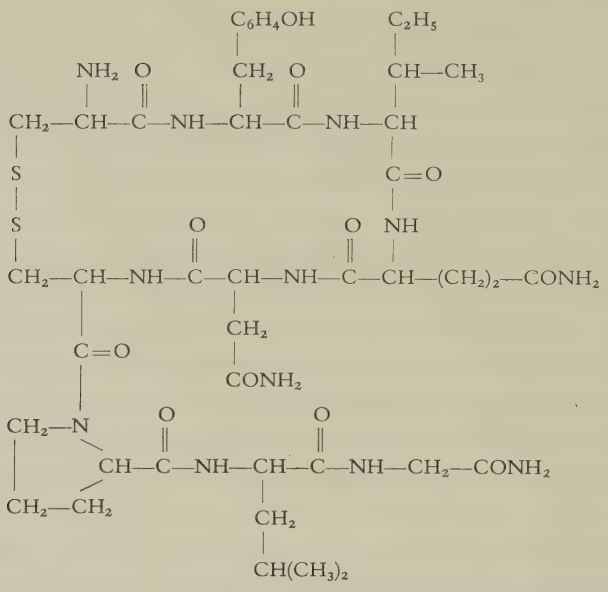

Fig. 3. Oxytocin.

tory on composition, molecular weight, terminal groups, as worked out by our use of the Sanger dinitrophenyl end group procedure[55], and on the cyclic structure involving the disulfide linkage which we had established, along with his independent studies on the sequence of amino acids involving partial hydrolysis with acid and with an enzyme. The interpretation of the data and the assumptions made were quite parallel in both laboratories.

As already mentioned, we felt that synthetic proof of the structure we had arrived at had to be adduced; that is, of course, if the synthesis were within the range of possibility. I might point out that the synthesis of this structure meant the synthesis of an octapeptide of eight different amino acids, one of them being cystine. In addition, this desired octapeptide contained three amide groups and was a cyclic polypeptide for which no known synthetic methods were available – a cyclic pentapeptide with a tripeptide side chain. To add to the challenge was the fact that we knew the final product was relatively unstable and easily inactivated. Gentle means would therefore have to be employed.

The clue to the synthetic approach to this compound rested on our work with Sealock, carried out in the 1930's, on the reduction of oxytocin and the oxidation of the reduced oxytocin without appreciable inactivation at either step, which has already been discussed[20]. On the basis of our postulated struc-

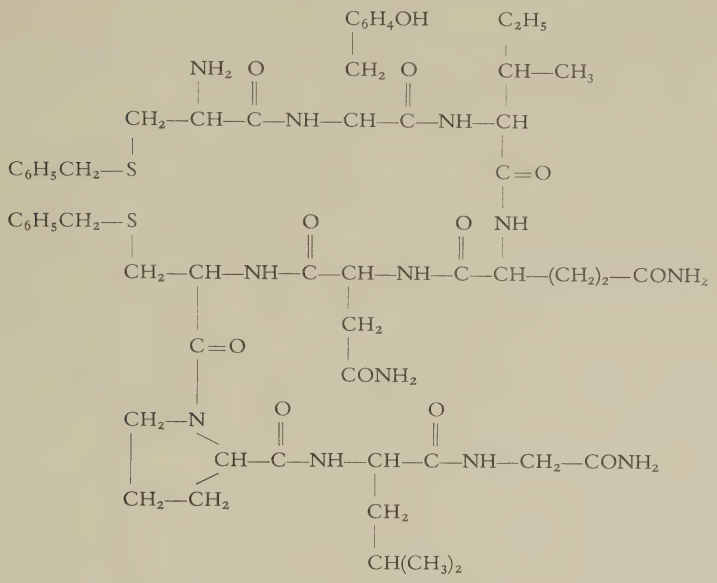

Fig. 4. Benzylated derivative of reduced oxytocin.

ture for oxytocin (Fig. 3), the reduction of oxytocin and subsequent oxidation could be interpreted as involving the opening and closing of the 20-membered ring. The reduced oxytocin would then have a linear structure containing two sulfhydryl groupings in place of the disulfide linkage in oxytocin. Furthermore, if the proposed structure for oxytocin were correct, reduction of oxytocin with sodium in liquid ammonia followed by addition of benzyl chloride should give rise to the *S*, *S'*-dibenzyl derivative of reduced oxytocin, possessing the structure shown in Fig. 4.

Since benzylation of reduced oxytocin had led to inactivation in the earlier study, the expectation was that this benzylated derivative of reduced oxytocin would be biologically inactive. With what we now knew of the structures involved, we could see no reason why treatment of the *S*,*S'*-dibenzyl derivative of reduced oxytocin with sodium in liquid ammonia should not lead to the biologically active, reduced oxytocin; oxidation of the sulfhydryl form by aeration should then lead to the regeneration of oxytocin itself, if our concepts and line of reasoning were valid.

We therefore decided to investigate the benzylation and debenzylation using highly purified oxytocin preparations in work with Gordon[56]. Our best sample of natural oxytocin was treated with sodium in liquid ammonia followed by the addition of benzyl chloride to the liquid ammonia solution.

From this reaction mixture, a product was obtained, which on amino acid analysis had the expected composition, containing two moles of *S*-benzyl-cysteine along with one mole of each of the other seven amino acids present in oxytocin.

This material was biologically inactive. The isolated dibenzyl derivative was then dissolved in liquid ammonia and metallic sodium was added. After removal of the ammonia, the product was dissolved in water and oxidized by passing air through the solution at a pH close to neutral; a biologically active product was obtained. From a comparison of the physical, chemical, and biological properties of the starting and regenerated material, we were convinced that oxytocin had been regenerated from its *S*,*S*′-dibenzyl derivative. With this result, I was confident that the door was opened to a synthetic attack on oxytocin.

If the linear dibenzyl nonapeptide shown in Fig. 5, which possesses a carbobenzoxy group on the amino group of one of the cysteine residues, could be synthesized, it should be possible to convert this protected nonapeptide by reduction to the reduced form of oxytocin and by subsequent oxidation to oxytocin (see Fig. 5).

The parallelism between this approach to the synthesis of reduced oxytocin and the approach to the synthesis of glutathione, which I have already discussed, is at once apparent. In both cases, the cysteine residue(s) present in the intermediates for the syntheses were protected by carbobenzoxy and benzyl groups, which were to be removed in the last synthetic step by sodium in liquid ammonia.

After a consideration of the many ways in which the synthesis of the intermediate for oxytocin might be approached, and after exploratory synthetic studies, it was decided to attempt to prepare the compound by combining the tetrapeptide amide, *S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, through appropriate means with L-isoleucyl-L-glutaminyl-L-asparagine to obtain the heptapeptide amide, L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, and to condense the latter with *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosine, thus forming the desired nonapeptide amide, and we concentrated on this line of approach. In this work I should like to acknowledge the splendid collaboration of Ressler, Swan, Roberts, and Katsoyannis[53,57].

In addition to the classical methods for the formation of peptides, other recently developed procedures were employed. We were able to utilize to particular advantage the contributions of Vaughan and Osato[58] and of An-

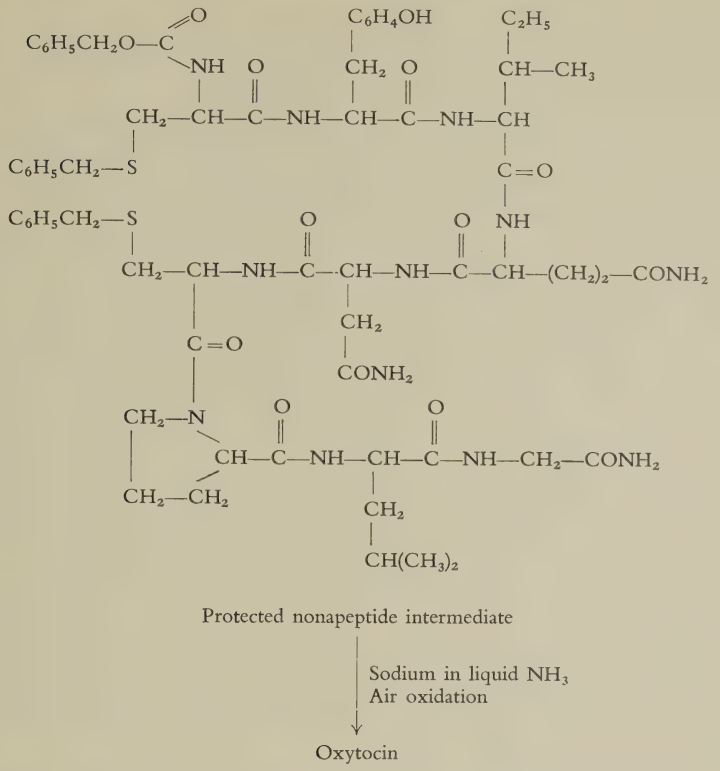

Fig. 5. Proposed intermediate leading to the synthesis of oxytocin.

derson, Blodinger, and Welcher[59]. In the former case, the mixed anhydride of an *N*-substituted amino acid, with, for example, isovaleric acid, is used for the preparation of the peptide in the latter method, tetraethyl pyrophosphite is the condensing agent.

In addition to using the carbobenzoxy group as a protective grouping for the amino group, we have also employed the tosyl group for this purpose. Removal of the tosyl grouping was accomplished by the use of sodium in liquid ammonia, as utilized in our work with Behrens[60] on the synthesis of α-amino-*N*-methyl-L-histidine. The tosyl group as a protective group in peptide synthesis was first used by Schoenheimer[61], who employed a hydriodic acid-phosphonium iodide cleavage.

As to the specific steps in our attempted synthesis of the hormone, the tetrapeptide amide was prepared with Ressler[53,57,62]. Ethyl carbobenzoxy-L-leucylglycinate was synthesized by the mixed anhydride procedure of Vaughan and Osato[58]. After catalytic removal of the carbobenzoxy group,

according to the procedure of Bergmann and Zervas[5], the ethyl-L-leucylglycinate was condensed with carbobenzoxy-L-proline, again by use of the mixed anhydride procedure with isovaleryl chloride.

After removal of the carbobenzoxy group by reduction with hydrogen in the presence of palladium catalyst, the ethyl L-prolyl-L-leucylglycinate was converted to the tetrapeptide, *S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycine via the dicarbobenzoxy-L-cystinyl derivative. Ethyl dicarbobenzoxy-L-cystinyl-bis(L-prolyl-L-leucylglycinate) was saponified in aqueous dioxane at $+5^{\circ}$ C in the presence of 1 equivalent of sodium hydroxide to yield the corresponding acid. The latter was converted to *S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycine according to our procedure for the removal of carbobenzoxy groups in cystine-containing compounds by the use of sodium in liquid ammonia[7] followed by benzylation[6] of the sulfur of the reduced compound in the same medium. The crude tetrapeptide was readily converted, with the use of benzyl alcohol and dry hydrogen chloride, to the crystalline benzyl ester hydrochloride, benzyl *S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinate hydrochloride. Amination was accomplished by allowing the free base obtained from the benzyl ester hydrochloride to stand in solution in methanolic ammonia for several days at room temperature. *S*-Benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide was isolated from its benzyl ester hydrochloride as a crystalline hydrate.

A novel method was devised for the synthesis of glutaminyl-asparagine with Swan[53,57,63]. Harington and Moggridge[64] had already shown that heating of tosyl-L-glutamic acid under reflux with acetyl chloride gave the mixed anhydride of 1-tosylpyroglutamic acid and acetic acid, which on hydrolysis in aqueous dioxane gave 1-tosylpyroglutamic acid, and that this compound with phosphorus pentachloride gave 1-tosylpyroglutamyl chloride. They had shown that the pyrrolidone ring in these compounds could be opened with aqueous alkali but was stable to anhydrous ammonia. In our work, tosyl-L-glutamic acid was treated directly with phosphorus pentachloride. The reaction product was then coupled with L-asparagine in the presence of aqueous magnesium oxide as the condensing agent[5] to give 1-tosylpyroglutamyl-L-asparagine. The latter compound with concentrated aqueous ammonia yielded tosyl-L-glutaminyl-L-asparagine, the pyrrolidone ring having been opened by addition of the elements of ammonia. Detosylation of the tosyl dipeptide by treatment with sodium in liquid ammonia gave L-glutaminyl-L-asparagine.

For the preparation of tosyl-L-isoleucyl-L-glutaminyl-L-asparagine with

Katsoyannis[53,57,65], tosyl-L-isoleucine was converted to the corresponding acid chloride and the latter was then coupled with L-glutaminyl-L-asparagine in the presence of magnesium oxide to give the tosyl tripeptide.

Condensation of this tripeptide with the tetrapeptide was effected by the use of tetraethyl pyrophosphite according to the general procedure of Anderson, Blodinger, and Welcher[59]. Removal of the tosyl group from the protected heptapeptide amide was effected with sodium in liquid ammonia, by which the benzyl group was also removed. The resulting reduced compound was then benzylated with benzyl chloride in the same medium to yield the heptapeptide amide.

Finally, the heptapeptide amide was condensed with *S*-benzyl-*N*-carbobenzoxy-L-cysteinyl-L-tyrosine[66], a dipeptide which had originally been prepared by Harington and Pitt Rivers[67]. The condensation was brought about in the presence of tetraethyl pyrophosphite to yield *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.

This was the compound we had set about to prepare as our proposed intermediate for the synthesis of oxytocin. If our structure for oxytocin were correct, we had every reason to believe that this compound, on reduction with sodium in liquid ammonia, followed by appropriate oxidation, would give us oxytocin (see Fig. 5).

This intermediate was therefore treated with sodium in liquid ammonia, by which both the benzyl and carbobenzoxy groups were removed. The reduced material obtained after evaporation of the ammonia was oxidized by aeration in dilute aqueous solution at pH 6.5 and tested for biological activity by the rat uterine strip method[68,69] and by the chicken vasodepressor method of Coon[70], which utilizes the property of oxytocin of lowering the blood pressure of the fowl and has been adopted by the United States Pharmacopoeia as the method of assay for oxytocin[40]. Based on the assumption that the synthetic material has a potency equivalent to that of the purified natural material (500 units per mg), the yield of biologically active material, as determined by the two types of assay, ranged in several runs from 20 to 30 per cent of the calculated amount from the protected nonapeptide intermediate. The yield approximated closely the yield of oxytocic activity obtainable under similar conditions from the *S,S'*-dibenzyl derivative of reduced natural oxytocin[56].

The purified synthetic product isolated by countercurrent distribution and our best sample of natural oxytocin were assayed against each other fol-

lowing rigorously the procedures outlined by the U. S. Pharmacopoeia. The results indicated that the activity of the synthetic material[53,57] was indeed very close to that of the natural oxytocin[33].

This synthetic material and natural oxytocin were then compared by a battery of tests – physical and chemical[53,57]. They both had the same amino acid composition. They showed, within experimental error, the same optical activity, partition coefficient (in two different solvent systems), electrophoretic mobility (at two different hydrogen ion concentrations), infrared pattern, ultraviolet spectrum, and effluent pattern from an IRC-50 resin[71].

The synthetic material formed an active flavianate which had the same crystalline form (fine, silky needles) and the same melting point as the flavianate obtained from natural oxytocin.

On treatment with bromine water, the synthetic material underwent the cleavage into two fragments encountered with natural oxytocin, both giving rise to β-sulfoalanyldibromotyrosine and a sulfonic acid heptapeptide[51].

Sedimentation studies on the molecular weight of natural oxytocin and the synthetic material were kindly made by Schachman and Harrington of the University of California. The natural and synthetic materials behaved identically, and the values obtained were in the expected range.

We were fortunate at this stage that we had the collaboration arranged with the Lying-In Hospital group of our Medical Center on the use of our highly purified natural oxytocin in induction of labor and in milk ejection, for the natural and synthetic material were then compared on human subjects. The synthetic product was fully effective in stimulating labor in the human and in milk ejection and could not be distinguished from the natural oxytocin in its action. Approximately 1 microgram of either the natural oxytocin or the synthetic material given intravenously to recently parturient women induced milk ejection in 20 to 30 seconds[43].

These comparisons of the physical, chemical, and biological properties of the synthetic product with those of the purified natural oxytocin justified in our estimation the conclusion that the synthetic octapeptide amide is oxytocin and that the structure shown in Fig. 3 represents that of the hormone[57].

With the establishment of the structure of oxytocin, a new ring system, that is, a 20-membered ring involving a disulfide linkage, was for the first time recognized in nature. Our studies have indicated that this same sized ring is present in vasopressin, the blood-pressure-raising and antidiuretic hormone of the posterior pituitary[72]. Recently it has been shown by Sanger

and co-workers[73] that a ring of the same size involving a disulfide linkage occurs in the insulin molecule, as part of a more involved structure.

The establishment of the structure of oxytocin and vasopressin will undoubtedly open the door to a better understanding of these hormones by the biochemist, the physiologist, the pharmacologist, and the clinician. Moreover, it should provide a suitable basis for the study of the relationship of chemical structure to biological activity in these protein-like substances. The synthesis of oxytocin will afford a means of obtaining the compounds necessary to the study of this relationship and may, in addition, point the way to the synthesis of more complex sulfur-containing polypeptides.

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Biography

Vincent du Vigneaud was born in Chicago, Illinois, on 18th May, 1901, the son of the late inventor and machine designer Alfred J. du Vigneaud and his wife, Mary Theresa. He studied under Professor C. S. Marvel at Illinois University and took his B.Sc. degree in 1923 and M.Sc. in 1924.

During the year 1924–1925 he was assistant biochemist to Dr. W. G. Farr at the Philadelphia General Hospital and served on the Staff of the Graduate School of Medicine of Pennsylvania University. In 1927 he worked with Professor J. R. Murlin and submitted a thesis to the School of Medicine of Rochester University which earned him the Ph.D. As a Fellow of the National Research Council he worked with Professor J. J. Abel at Johns Hopkins University Medical School, with Professor George Barger at Edinburgh University Medical School and with Professor Charles R. Harington at London University College Hospital.

On his return to America, du Vigneaud joined the Physiological Chemical Staff at Illinois University under Professor W. C. Rose and in 1932 he became Head of the Biochemistry Department at the George Washington University School of Medicine. The Cornell University Medical College offered him a Professorship as Head of the Biochemistry Department in 1938.

Du Vigneaud has held many lectureships in universities in the United States and England, among the latter the Liversidge Lectureship at Cambridge, and in the summer of 1947 he was Visiting Lecturer of the American Swiss Foundation for Scientific Exchange in Switzerland. His Messenger Lectures at Cornell University in 1950 were published in 1952 as *A Trail of Research in Sulphur Chemistry and Metabolism and Related Fields*. Many learned chemical societies in America have conferred awards on du Vigneaud and he received the Chandler Medal of Columbia University in 1955 and the Willard Gibbs Medal of the American Chemical Society a year later. Honorary science doctorates were bestowed on him by New York and Yale Universities in 1955, and by Illinois University in 1960.

Honorary fellowships have been conferred on du Vigneaud by the Royal

Society of Edinburgh, the Chemical Society and the Royal Institute of Chemistry, London. He has been elected to membership of many scientific academies among the most noteworthy being the Board of Trustees of the Rockefeller Institute and the National Institute of Arthritis and Metabolic Diseases.

His researches have centred mainly about sulphur-containing compounds of biochemical importance, being concerned originally with the sulphur of insulin and more recently with two hormones of the posterior pituitary gland – oxytocin and vasopressin. He has also studied intermediary metabolism, amino acids and peptides, transmethylation and metabolism of one-carbon compounds, transsulphuration, biotin and penicillin.

Du Vigneaud married Zella Zon Ford in 1924; they have a son, Vincent, Jr.(b.1933) and a daughter, Marilyn Renée (b.1935).

Chemistry 1956

Sir CYRIL NORMAN HINSHELWOOD

NIKOLAI NIKOLAEVIČ SEMENOV

«for their researches into the mechanism of chemical reactions»

Chemistry 1956

Presentation Speech by Professor A. Ölander, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Your Royal Highnesses, Ladies and Gentlemen.

The Nobel Prize which is now to be given to Sir Cyril Norman Hinshelwood and Academician Nikolai Nikolaevič Semenov «for their researches into the mechanism of chemical reactions» reminds us of the very first Nobel Prize in Chemistry, which was awarded in 1901 to the Dutchman Jacobus Henricus van 't Hoff. He received his prize for «the discovery of the laws of chemical dynamics», i.e. the velocity of chemical reactions.

Van 't Hoff and the Swede Svante Arrhenius had already in the 1880's disclosed that when molecules of two substances collide, the collision must be sufficiently violent if the initial molecules are to break down and their atoms to rearrange into new molecules, that is, for a chemical reaction to take place.

Thirty years ago Hinshelwood studied a number of chemical reactions which allowed him to draw important conclusions concerning the collisions between molecules, which set them in such vibration that they became unstable.

There are some chemical reactions which are extremely sensitive to light. In 1900 Max Planck had found that light was composed of discrete quanta. It was then natural to think that when a light quantum hits a molecule, it could be excited in such a way that it underwent a chemical reaction. But how could one possibly understand that a single absorbed light quantum could cause perhaps a million molecules to react?

In 1913 the German chemist Max Bodenstein put forth an idea which proved to be extremely fertile, the idea of chain reactions. This means, that if two molecules react, not only molecules of the final reaction products are formed, but also some unstable molecules, having the property of being able to react with the parent molecules without the collision being very violent. In this reaction, new unstable molecules are formed besides stable reaction products and so on. We thus obtain a chain of reactions, so when two molecules have reacted, they cause a great number of more molecules to react.

A Danish and a Dutch scientist, Christiansen and Kramers, in 1923 pointed

out that such a chain reaction need not start with a molecule excited by light, but could also start with two molecules colliding violently in the way Van 't Hoff had thought of.

Christiansen and Kramers also set forth another fruitful idea. If in one link of the reaction chain not only one, but two or more unstable molecules are produced, the reaction chain will branch. The result is that the reaction will spread over the whole mixture so it reacts in its entirety extremely rapidly, thus giving rise to an explosion. However, they did not elaborate the idea further, but pursued other researches.

The laws that were advanced by Van 't Hoff do not always seem to be valid, a fact which he was well aware of himself. An example: phosphorus glows in the dark, because it is oxidized by the oxygen of the air. We should then expect phosphorus to glow five times more intensely if we put it in pure oxygen, as air contains only 20% of oxygen. But already in the 18th century it was known that phosphorus did not glow at all when lying in pure oxygen. The oxidation also ceases suddenly when the oxygen content decreases below a certain limit.

The combustion of phosphorus vapour and oxygen was studied in 1926 by two scientists in Leningrad, Chariton and Valta. The greatest authority of that time on chemical reaction velocities, Bodenstein, whom I just mentioned, said frankly that their results were incomprehensible and must be wrong. They *were* incomprehensible from the point of view of that time, but the essential results were *not* wrong. Semenov reinvestigated the matter and found that it really was so that a mixture of phosphorus vapour and oxygen did not react at all if the gas pressure was too small or too great, but that at intermediate pressures the mixture exploded. Semenov disclosed that the idea of Christiansen and Kramers gave the explanation of this behaviour. He and his team could show, that the pressures, at which the mixture exploded, were dependent on the proportion of gases and dimensions of vessel in a way which agreed completely with the assumption that this combustion was a chain reaction.

The mathematical relations in this case were rather simple. There are other combustions with far greater practical importance, but which are much more complicated. I will first mention the combustion of hydrogen with oxygen. This important reaction was studied both by Hinshelwood and his team in Oxford and by Semenov and his team in Leningrad. Of course also many other scientists have contributed to the final elucidation, but the present prize-winners have indicated the principles guiding the work. Another

technically important chain reaction is the combustion of carbon monoxide, not to mention the combustion of hydrocarbons.

When it was found that a great number of reactions were chain reactions, many people in the first enthusiasm thought that almost all reactions were chain reactions and that the simpler mechanisms previously thought of were exceptions. But Hinshelwood put the matter in order. He found substances which could simultaneously react in two ways, one part reacting by a chain mechanism and at the same time the rest reacting in the old-fashioned way.

There are many reactions which do not start at once when the substances have been mixed, but after a while. A number of explosions behave that way and are therefore extremely unpleasant. Semenov showed that the concept of chain reaction could also explain this behaviour.

I shall conclude with an example showing how these researches have a direct bearing upon our daily life. I have just mentioned that the combustion of hydrocarbons is a chain reaction. When internal combustion engines were built with higher and higher compression in order to get more energy out of them, the result was that the air–gasoline mixture burned much more rapidly than was appropriate, it exploded. But the rapid combustion could be regulated by using gasoline containing suitable hydrocarbons or with suitable additions. Such gasoline is said to have a high octane number. The vast amount of work carried out in recent years to augment the effect of internal combustion engines in this way is founded, as far as its chemical aspects are concerned, on the fundaments laid down by these prize-winners.

Sir Cyril Norman Hinshelwood, Academician Nikolai Nikolaevič Semenov. More than half a century has elapsed since the first Nobel Prize in Chemistry was given to Van 't Hoff for his discovery of the laws of chemical dynamics. Some of the greatest advances in chemical kinetics since that time have emerged from your researches and they have inspired a great number of scientists to continued fruitful studies. Your results are of equally great importance to technology and to the more theoretical aspects of chemistry.

On behalf of the Royal Swedish Academy of Sciences I wish to extend to you our warmest congratulations. May I now ask you to receive the Nobel Prize for Chemistry for the year 1956 from the hands of His Majesty the King.

Cyril N. Hinshelwood

Chemical kinetics in the past few decades

Nobel Lecture, December 11, 1956

The task before me is to speak of the evolution during the last few decades of the branch of physical chemistry which treats of the intimate mechanism of chemical reactions. The nature of the present occasion seems to call for a survey taken from a rather more personal point of view than is normally desirable or proper. Since the Swedish Academy of Sciences has done me the great honour of bringing me to Stockholm to receive their high award, it is perhaps suitable that I should give some account of the lines of thought and of the accidents which have determined the course of the work in which, with others, I have taken part.

Nobody, I suppose, could devote many years to the study of chemical kinetics without being deeply conscious of the fascination of time and change: this is something that goes outside science into poetry; but science, subject to the rigid necessity of always seeking closer approximations to the truth, itself contains many poetical elements.

There are many ways in which the mysteries of chemical change might begin to impress themselves on a young chemist. I will mention one: it belongs to practical life and is a real example. Explosives have to be tested for stability and this is done by measuring their decomposition at a suitably high temperature. Phenomena of great interest appear, revealing a remarkably large influence of temperature, a great acceleration of the rate if the solid liquefies, and various striking catalytic effects. Many major problems present themselves, which can be roughly summed up as those involving the dependence of chemical change upon the energy and the environment of the molecules. It was obvious that these problems offered many mysterious and fascinating aspects. In fact they prove to contain more than enough for a lifetime's study.

In the early twenties one of the central questions in physical chemistry was that of unimolecular reactions, in which interest had been newly roused by the idea that such reactions were provoked by the absorption of radiation. A curious circumstance is that much theory was centred on the category of unimolecular reactions in the gas phase, of which in 1920 no authentic ex-

ample was actually known. The much discussed decomposition of phosphine proved to take place on the vessel walls, a fact linking up with the renewed interest in heterogeneous catalysis of gas reactions which the work of Langmuir on reactions with heated filaments had awakened. Against this background work in Oxford became directed to two ends. First, the discovery of new homogeneous reactions, and in particular those of single molecules, and secondly, the study of the factors which made the mechanism of heterogeneous catalysis often the preferred one.

The study of the heterogeneous reactions revealed one principle, which now seems trite enough, but was very stimulating in the days when molecular mechanisms of chemical changes were largely wrapped in mystery, namely that the affinity of the solid catalyst for part of the reacting molecule opens a reaction path with lower activation energy. Examples of homogeneous changes multiplied, and a new field opened up when thermal decompositions of organic vapours such as acetone, ether and acetaldehyde proved a rich source of unimolecular reactions.

Like the famous example of the nitrogen pentoxide dissociation, investigated by Daniels and Johnston in 1921, all these reactions eventually proved to have mechanisms of a much more complex character than was at first supposed. Two major findings have, however, stood the test of time. First, there was the unmistakable evidence that molecular collisions play the all-important role in communicating the activation energy to the molecules which are to be transformed and, secondly, there was the recognition of the activation energy (derivable from the Arrhenius law of temperature dependence) as a dominant, though by no means the sole factor determining reactivity in general.

These things helped to give shape and coherence to the whole subject of chemical kinetics. While the development of these themes was being pursued, a new one entered to add complexity to what was already complex enough, the theme namely of chain reactions.

The principle of chain propagation had been enunciated by Nernst to explain large departures of photochemical reactions from Einstein's rule, and the possibility of its intervention in thermal reactions had been suggested by Christiansen. The first clear evidence that chain processes play a major part in thermal reactions in the gaseous phase came from the investigation of the unexpected properties of such reactions as the formation of water from its elements, and the oxidation of hydrocarbons like ethylene. Not only were the relations between rate and concentration inexplicable in terms of simple

applications of the mass action law to non-chain processes, but the reactions showed a quite surprising dependence upon the size of the containing vessel, the presence of what should have been chemically inert gases, and the presence of sensitizers or inhibitors.

The reaction $2H_2 + O_2 \rightarrow 2H_2O$, from one point of view perhaps among the most elementary in chemistry, proved to offer a wealth of complex and intriguing behaviour – not all details of which are even yet fully understood. Victor Meyer had thought it an interesting reaction to study, but found it, apparently, intractable. Bodenstein showed that at certain temperatures the union took place on the walls of the vessel. The work in Oxford started from the simple idea that in the region of temperature between that used by Bodenstein and the inflammation temperature homogeneous processes must come into play.

The now well-known phenomena of the lower and upper explosion limits were observed. Semenov, then in Leningrad, had just explained the sharp transition from negligibly slow reaction to inflammation (a «lower limit») of phosphorus vapour by the theory of chain branching. It was soon clear that the lower limit with hydrogen and oxygen was of a similar nature. The branching was shown here to be controlled by the deactivation of chain carriers, at the vessel wall, and the « upper limit » was proved to depend upon the removal of chain carriers by three-body collisions in the gas phase.

The study of the hydrogen–oxygen reaction was the first point at which the work in Oxford came into close contact with that of Semenov. Our indebtedness to his ideas was at once recognized, and the early exchanges opened friendly relations between Semenov and myself which have lasted ever since. I should like at this point to mention also my personal debt to an earlier great pioneer of chemical kinetics, Max Bodenstein, who himself plays a part in the curious history of the hydrogen–oxygen reaction.

The phenomena involved in the initiation, propagation and termination of chains are now among the commonplaces of physical chemistry. Each process may be separately influenced by the conditions, so that the variety and complexity of the experimentally observable effects can no longer be wondered at. The detailed explanation of numerous examples continues to be of importance especially in connexion with the control of the polymerization reactions widely used in the modern plastics industry.

The importance of thermal decomposition reactions of organic molecules in connexion with the fundamental theory of chemical reactions has already been mentioned. Once chain processes were discovered it was obviously

necessary to know whether they were involved in these thermal decomposition processes. If so, the rather elaborate theoretical studies for which some of the results had been used would need considerable revision. A decision on this point was not quite simple. Work on the occurrence of free atoms and radicals, notably by R. W. Wood and Paneth, did indeed suggest the possibility of their intervention in such reactions as the ether decomposition, and Rice and Herzfeld formulated theoretical reaction schemes on this basis. On the other hand, tests for the actual presence of free radicals in the reacting systems which Patat and Sachsse made by the application of the o–p hydrogen method seemed at first to negative the idea.

Definite evidence was at length forthcoming when Staveley and Hinshelwood began to study the influence of nitric oxide on these reactions. The idea which initiated the work was simply that an odd electron molecule such as NO might well produce some interesting effects. Quite how interesting the effects would prove was not altogether foreseen. The pronounced inhibition caused by relatively minute quantities of this substance in a whole series of reactions which could conceivably involve the intervention of alkyl radicals left no doubt that the radical chain mechanism was in fact operative.

The working out in full detail of the mechanism of the large number of types of chain process now constitutes an extensive and elaborate chapter of chemistry which can absorb the attention of many workers for quite a long time to come.

The prevalence of chain mechanisms greatly complicates the problem of establishing a really complete and detailed theory of unimolecular reactions. Although a chain decomposition is often enough initiated by a unimolecular step, the observable kinetic relations are so much complicated by the varying possibilities for propagation and termination that definite conclusions about the details of the initiating process itself are very hard to arrive at. Some reactions, it is true, have been shown to be unimolecular without the participation of chains, but they are rare, and although the study of them is of great importance it deals with a small minority of the examples presented by Nature.

Great interest, therefore, attaches to the following problem. With increase in the amount of nitric oxide added to a system such as decomposing butane the rate of reaction falls not to zero but to a definite limit. This limiting rate has been widely regarded as that of a unimolecular non-chain reaction, that is to say, the dissociation of the butane into its stable products without the liberation of free radicals. If this view is correct the butane decomposition,

as well as a large number of other examples, can be used in the study of unimolecular reactions, provided that enough nitric oxide is added to suppress the chains.

But this view about the limiting rate is not without uncertainty. The alternative view is that the limit is determined by a steady state in which the nitric oxide starts as many chains as it stops

$$\begin{aligned} NO + RH &\rightarrow\!\!\!\!\rightarrow NOH + R \\ R + RH &\rightarrow\!\!\!\!\rightarrow \text{chain} \\ R + NO &\rightarrow\!\!\!\!\rightarrow \text{end} \end{aligned}$$

The arguments on the two sides are clear enough. That the limit corresponds to a molecular reaction is suggested by the facts

(a) inhibitors as different as nitric oxide, propylene and isobutene give just the same limit (though quite different amounts of them are needed to reach it);

(b) the addition of certain foreign gases such as sulphur hexafluoride and carbon tetrafluoride accelerates the «residual» reaction in presence of nitric oxide by precisely the same absolute amount as they accelerate the much more rapid reaction occurring in the absence of the inhibitor – a fact suggesting that the uninhibited chain reaction and the residual reaction are independent entities;

(c) the kinetics of the «residual» or «maximally inhibited» reaction show certain peculiarities which they share with other unimolecular reactions (notably the decomposition of nitrous oxide and the dissociation of di-*tert.*-butyl peroxide), known not to involve chains at all. The kinetic form would not be easy to interpret by the hypothesis of an NO-induced chain reaction – though it would be rash to assert that this could not be done with suitable ingenuity.

The argument on the other side is based chiefly upon the fact that deuterium exchange in such systems as C_2H_6—C_2D_6 proceeds to about the same proportional extent whether the main decomposition is damped down by nitric oxide or not.

Although Rice and Varnerin reached this conclusion Wall and Moore had found that the exchange was reduced by the addition of an amount of nitric oxide which was not sufficient to slow the decomposition to its limiting rate, and Stephen and Danby found that nitric oxide would in fact inhibit all the gas phase chain reaction in the simple system $D_2 + H_2 \rightarrow\!\!\!\!\rightarrow 2HD$.

At present my own support is for the view that the residual reaction is molecular, and that the experiments on deuterium exchange have other explanations. This may, of course, prove to be erroneous. If so, Nature has played a curious trick in arranging the coincidence whereby such varied agents can both start and stop chains with efficiencies which remain in a constant ratio. If this coincidence really does occur, then there is also a very puzzling problem to be solved in explaining the kinetic relations actually found with the maximally inhibited reactions themselves.

It is a rather strange position to defend a certain view because the stronger arguments seem to be in its favour, and yet clearly to realize what arguments there are on the other side. Here, ironically enough, I may be wrong for maintaining that there is a non-chain component in some reactions which until the advent of the nitric oxide method could not be certainly proved to involve any chains at all. There is perhaps a moral in this to which I shall refer at the end. In the meantime all kinds of detailed experiments are still going on to resolve this problem.

Turning now to another question which has exercised attention from the very beginning, there are still puzzling and intriguing aspects about the value of the constant A in the equation which relates the rate constant of a unimolecular reaction to the activation energy

$$k = Ae^{-E/RT}$$

The processes by which the isolated molecule undergoes its first split, whether this is to be followed by a chain or not, seem to present a special interest both to theoretical and practical researchers. As we know, communication of energy by collision initiates a sequence which may be written as follows

	(1)		(2)	critically	(3)	
activation	$\rightarrow$	energized	$\rightarrow$	activated	$\rightarrow$	products
by collision	$\leftarrow$	molecules		molecules		
	(1′)					

At low pressures 1 determines the rate. At higher pressures 1 and 1′ are nearly in equilibrium. According to the simplest theoretical picture of the process, evolved by Rice, Ransperger, Kassel and others and more recently greatly refined by Slater, the critical act in the whole sequence is 2 which

consists simply in the accumulation into one special bond of enough energy to rupture it. The mathematical analysis of this idea leads to the conclusion that A is of the order of magnitude of a molecular vibration frequency.

It sometimes is of this magnitude – a refreshing but not too common example of Nature's conforming to a simple pattern of behaviour. (Incidentally, a good many of the «inhibited» paraffin decompositions, whose questionable status as primary unimolecular processes has been mentioned, conform very well.

In other examples A exceeds any likely vibration frequency by several powers of ten. Here it would certainly seem that the simple picture of a critical concentration of energy into one bond is inadequate, and I believe that, as a general proposition, this conception must be widened. The whole idea of the energy relations in unimolecular reactions has had quite an eventful history. First, the idea that the activation energy could not be communicated by collision had to be disposed of. Next, until it was realized that energy could be, as it were, mobilized from numerous internal degrees of freedom (in the process indicated by 2 of the above scheme) there were misgivings about the adequacy of possible rates of energy transfer (and several writers even entertained the possibility that energy conservation did not apply in such processes). Then came the rather oversimplified view I have just mentioned, which is in course of being widened, so that we may now envisage many alternative states of energy distribution from which the final transformation may take place.

Another interesting set of problems has arisen out of the scheme of things set out above. Process 1 is certainly rate-determining at the lowest pressures, and process 2 takes over at higher pressures. Where one rate-determining process gives way to another the characteristic plot of the first order rate constant, k, against the pressure shows a change of slope. In several examples there are very strong indications that such transitions occur in more than one region of pressure. In other words, there are still further changes in the nature of the rate-determining process. The obvious interpretation is that process 2 involves further steps or even that process 3 may be collision-dependent. In the example of the decomposition of nitrious oxide there is a considerable probability that the extra process introduced into the sequence is that of

$$N_2O \text{ singlet} \rightleftarrows N_2O \text{ triplet}$$

These matters are still subjects of research.

I must turn now to a quite different field of investigation, that namely of the relation between reactivity and structure. Here, perhaps, we may distinguish two main groups of problem. The first is one of a rather broad, indeed almost philosophical character. In the equation for a reaction rate constant

$$k = Ae^{-E/RT}$$
$$\text{or } \ln k = \ln A - E/RT$$

$\ln k$ is the kinetic analogue of a free energy, E of a total energy and $\ln A$ of an entropy. E does indeed represent the energy required to lift molecules to the transition state, A is concerned with the probability that all other requisites for reaction are fulfilled. Structural changes in reacting substances can affect both A and E. Sometimes, as is beautifully exemplified by the benzoylation of a whole series of aniline derivatives containing different substituents, only E is affected. Sometimes, however, there is a deep inner correlation between the changes in E and the changes in $\ln A$, the completer understanding of which is now being sought, and it is to be hoped may reveal some more of the fundamental harmonies of Nature.

The other great groups of problems is that of the relation between changes in rate, whether expressed through E, $\ln A$ or both, and electron displacements within molecules, the theory of which has been largely developed by organic chemists, and only very partially interpreted in terms of the quantum mechanical theory of valency.

Perhaps as typical of the interesting relations in the structural field I may mention one or two examples. The oxidation of hydrocarbon vapours, a very typical chain reaction, offers a rather remarkable one. The oxidation rate for *n*-octane is many powers of ten greater than that of ethane, and detailed investigation shows the major influence governing the variations to be a most astonishing stabilising influence of methyl groups which strongly discourage attack at any point of a hydrocarbon chain adjacent to them. This is indeed an elegant problem for theoretical structural chemistry.

Here are two more such problems for modern valency theory which experiment presents. In a reaction involving a benzene derivative the rate is influenced by substituents in the ring, but to widely varying extents in different examples, and intriguing questions arise about the factors determining the ease of transmission of the substituent influence to the reaction-centre. The benzoylation of aniline is slowed down hundreds of times by a nitro-group, while the dissociation of nitrobenzaldehyde bisulphite compound differs little in rate from that of the parent substance.

When several substituents are introduced into aniline their effects on the free energy of activation of the benzoylation are almost strictly additive: in other examples the additivity is not so nearly so evident, offering once again an interesting exercise for the theoretical chemist.

Perhaps I am beginning to indicate too many questions and too few answers, but to do this at least re-creates the general atmosphere of this whole subject.

I am now going to turn briefly to a still more mysterious field, that of the living cell.

With a subject like chemical kinetics whose ramifications and details multiply without end, the individual worker is always confronted with the choice whether to repeat, perfect, amplify and consolidate, or whether to press forward into the completely unknown. In one respect at any rate a small group in Oxford has pursued the second course. The direction of this further exploration has been to seek an understanding of some of the kinetic phenomena of the living cell.

In some chemical systems, specially suitable for illustrating the elementary principles of kinetics, simple one-stage systems are met with, though less rarely than might have been hoped. In most of the systems, easily accessible to experiment reactions proceed by more or less complicated combinations of steps. In the living cell the multiplicity of reactions is not just an unfortunate complication, but the essential condition for the phenomena of life to be possible at all.

Suppose we liken individual chemical reactions to so many simple musical themes each played on a different instrument, then the operation of a living cell depends upon the combination of all these elements into a symphony. Knowing something of the theory of the simple elements can we find something about the rules of composition of the symphony?

To discuss properly the idea contained in this question would require not a small part of one lecture, but a whole series of lectures. All I can do is to indicate certain facts and observations the following up of which brings us into contact with the major question.

Bacterial cells (and some other unicellular organisms) grow in very simple media at defined rates. They synthesize, by long sequences of reactions, all the complex components of their structure. When the chemical environment is changed the growth rate alters. Gradually, however, by what is called an adaptive process, the cells undergo an internal reorganization in such a way that the rate, which may initially be very small, rises to a steady maximum.

The detailed study of this process gives interesting information about the way in which the chemical reactions are linked into what we term the reaction pattern of the cell.

Inhibitory substances, such as drugs, frequently impede growth very seriously, but gradually the cells appear to learn to overcome this inhibition. Detailed study of this process shows it to be capable of a kinetic explanation about which, needless to say, some biologists are sceptical but which helps to build up the picture of the cell's internal economy, and to show how much this is determined by kinetic principles.

There are many other problems which can be envisaged in terms of the reaction pattern of the cell. Cells contain numerous specific enzymes. The way in which these are built up as the cells multiply has some curious aspects, of which the explanation is certainly linked with the mode of coordination of the reaction pattern. When cells are provided with nutrient they grow and divide. The division seems to be determined by the moment at which a more or less critical amount of deoxyribose nucleic acid acid has been built up. Here is a fact relating kinetic phenomena closely to structural considerations.

I can not give you a full and coherent account of these things, but I mention them to show that there are plenty of mysteries left to explore, and also to suggest that kinetics can play a part in explaining some phenomena of that fascinating entity, the living cell. Even if I am wrong about this, it will have been worth the trouble to prove it.

One of my colleagues, Professor G. Temple, has drawn an interesting distinction between the « classical » and the « romantic » in scientific research. The former is exemplified by the demonstration of clear-cut and unquestionable mathematical propositions, or the devising and application of crucial logical tests of alternatives, the latter by the exploration of the unknown and the formulation of provisional hypotheses from incomplete data which need continual revision as the search proceeds. There is little doubt to which class the study of chemical kinetics belongs. Indeed some of us may well feel in retrospect like Dante

Mi ritrovai per una selva oscura
che la diritta via era smarrita.

I found myself in a dark wood,
where the straight way was lost.

And, indeed, I mentioned earlier an example of a situation where one or other of two quite respectable lines of argument must eventually prove to be in error. Is there a moral to be drawn? I am not sure, though we may perhaps console ourselves with the famous words of Lessing:

Nicht die Wahrheit, in deren Besitz irgendein Mensch ist oder zu sein vermeinet, sondern die aufrichtige Mühe, die er angewandt hat, hinter die Wahrheit zu kommen, macht den Wert des Menschen.

Or as an English writer has said « To travel hopefully is a better thing than to arrive ».

Biography

Sir Cyril Norman Hinshelwood was born in London on June 19, 1897. He was educated at Westminster City School and Oxford University where he gained Master of Arts and Doctor of Science degrees. He held successive fellowships at Balliol, Trinity, and Exeter Colleges; he was tutor of Trinity College from 1921 to 1937 and since 1937 he has been Dr. Lee's Professor of Chemistry, University of Oxford. He is a delegate of the Clarendon Press and he has served as a member of several Advisory Councils on scientific matters to the British Government. He was elected Fellow of the Royal Society in 1929, serving as Foreign Secretary from 1950 to 1955, and as President from 1955 to 1960. He was knighted in 1948 and appointed to the Order of Merit in 1960.

His early studies of molecular kinetics led to the publication of *Thermodynamics for Students of Chemistry* and *The Kinetics of Chemical Change* in 1926, the latest (fourth) edition of the latter appearing in 1940, and he subsequently worked on chemical changes in the bacterial cell, producing physicochemical explanations for the biological responses of bacteria to changes in environment. His findings proved to be of great importance in later research work on antibiotics and therapeutic agents, and his book on the topic *The Chemical Kinetics of the Bacterial Cell* was published in 1946. He has contributed a great number of original papers and reviews to journals of learned societies and to other scientific periodicals, and his latest book, *The Structure of Physical Chemistry* appeared in 1951.

Sir Cyril was President of the Chemical Society from 1946 to 1948, and President of the Faraday Society from 1961 to 1962, and included amongst the many awards he has gained are Lavoisier Medal, Société Chimique de France, 1935; Davy Medal, Royal Society, 1943; Royal Medal, 1947; Longstaff Medal, Chemical Society, 1948; Guldberg Medal, Oslo University, 1952; Faraday Medal, 1953; Avogadro Medal, Accademia dei Lincei, Rome, 1956; and Leverhulme Medal, Royal Society, 1960.

Honorary degrees conferred on Sir Cyril include Doctor of Civil Law (Oxford) and the Doctor of Science degrees of Bristol, Cambridge, Dublin,

Hull, Leeds, London, Sheffield, Wales, and Ottawa Universities. He holds honorary memberships of the major scientific societies of many countries.

Sir Cyril is unmarried. He is fluent in many languages and his main hobbies are painting, collecting Chinese pottery, and foreign literature.

Nikolai N. Semenov

Some problems relating to chain reactions and to the theory of combustion

Nobel Lecture, December 11, 1956

I should like to report here on researches into the field of chemical kinetics and the processes of combustion conducted over the last 20–30 years, firstly in my laboratory at the Leningrad Physical-Technical Institute and then in the Institute of Chemical Physics in the Academy of Sciences. These researches represent the outcome of many years' endeavours by a large group of scientific collaborators, many of whom have now become well-known authorities and leaders in individual branches of scientific learning. There can be no doubt that it is indeed thanks to the joint work, thanks to the continual mutual help, and at the same time thanks to the personal initiative of some members of our group that we succeeded in achieving the results which have been valued so highly here.

Successful exchanges of views and friendly discussions with learned men in other countries, particularly with Sir Cyril Hinshelwood, contributed much to the development of these researches and, particularly in the initial stage, to the development of new ideas. From our own experience, we have come to the conclusion that only the joint activity of men of learning in various countries can create conditions under which science can achieve successful further development.

Our investigations can be divided into two different groups, which are however closely linked with each other:

(1) the application of chemical kinetics to problems relating to the theory of combustion and explosion processes;

(2) the mechanism of chemical reactions, particularly chain reactions.

Every combustion or explosion process is above all a chemical reaction between the components of the combustible mixture, accompanied by the liberation of heat and various kinds of motion in gases. For this reason, all phenomena which occur in the combustion process are closely linked with the ideas and laws of chemical kinetics, and above all with the velocity of chemical reaction under non-isothermal conditions. According to Arrhenius' law, the heat generated by the reaction itself increases the velocity of

reaction. The existence of such retroaction is to the greatest possible extent characteristic of most phenomena of the combustion process.

As a result of the reciprocal effect of the reaction on the generation of heat in the mixture on the one hand and the increase in reaction velocity due to this greater heat, as already mentioned, on the other, a self-accelerating surge of heat is set up which we term auto-ignition. The dissipation of heat on the walls of the vessel renders the development of a heat surge possible only under certain conditions which are therefore all-important to the temperature of this auto-ignition. This temperature, which is a relative value (and by no means a constant of the substance, as was previously assumed by some authorities), is a function of the kinetic and thermal parameters, namely of the order of reaction, the activation energy, the density and composition of the combustible mixture, the heat of reaction and the dissipation of heat on the vessel walls.

Over the years 1928–1940, we gave[1] a mathematical formulation of the heat theory of auto-ignition, the conclusions of which were subsequently tested on many occasions experimentally, initially in the USSR and then in other countries. If the constants of reaction velocity and conditions of heat dissipation were known, it was possible to calculate beforehand the temperature of auto-ignition (flashpoint) at various pressures. The heat theory made it possible for any reaction of the auto-catalytic type, of which the kinetics were known, to calculate in advance the time of the explosion delay (sometimes as much as several minutes or even hours), as a function of the temperature and pressure.

The theory of normal flame propagation is based on the idea that the burning layer ignites adjacent cold layers due to the dissipation of heat. In earlier theories, it was assumed that the neighbouring layers ignited when the latter were heated to the temperature of auto-ignition, or flashpoint. However, this disregarded the chemical peculiarities of the process, in fact the chemical kinetics, which was not right and was in itself contradictory. Immediately after the excellent research of B. Lewis and G. von Elbe[2] into flame propagation in ozone (the authors did not indicate the temperature of auto-ignition but did take into account the diffusion of the fresh gas in the flame area), our group succeeded in developing a general heat theory for flame propagation in gases[3], and also a theory regarding the combustion of liquid and solid secondary explosives[4]. This theory provided a possibility of calculating the normal rate of flame propagation as a function of purely kinetic and thermal parameters. We tested this theory subsequently against

the combustion of carbon monoxide and oxygen mixtures and the combustion of liquid nitroglycol.

Soon after, the theory of the limits of concentration of normal flame propagation[5], a phenomenon so important to safety techniques, was also forthcoming. We explained this phenomenon by describing how the heat losses on the walls of the pipes in which the flame is propagated reduce the flame temperature and the rate of propagation; the lessening in the propagation rate, however, naturally leads to an increase in the heat losses. Considering this retroaction mathematically, it follows that the rate of propagation cannot be less than a certain value, which explains the existence of concentration limits in the propagation of flames.

The part played by mechanical motion, particularly turbulence in the gas, during flame propagation has long been noted. It was demonstrated however with particular clarity in our institute[6]. For example, it was shown that, if a wire spiral is applied along the inner wall of a glass tube containing a mixture of carbon monoxide and air, the rate of flame propagation in this mixture is approximately one thousand times greater than it is in the same glass tube but without the spiral of wire. The theory of turbulent combustion developed by G. Damköhler[7] and by the colleagues in our institute[8] is based on the idea that the surface of the flame front increases by virtue of pulsations. The overall flame propagation rate is determined in such cases largely by the square root of the quadratic mean of the pulsation rate, which means that it increases with increasing turbulence.

If the flame is propagated in tubes of sufficient length, it is accelerated due to turbulence in the fresh mixture. This acceleration of the flame produces a detonation condition in the combustion, ignition occurring due to the high temperatures generated due to compression of the gas in the percussion wave. In large diameter tubes, the detonation rate in mixtures bordering on the stoichiometric and having adequate reaction heat, does not depend upon the kinetics of the reaction, in accordance with the traditional theory of detonation, which allows of instantaneous combustion of the substance in the surge front. This theory cannot however explain why low-energy mixtures (diluted particularly by inert gases or mixtures at low pressure) do not detonate at all, why there are limits to concentration of detonation, why high-energy mixtures do not detonate in narrow tubes, etc. The dependence of the capacity for detonation upon chemical kinetics was demonstrated with particular clarity in our institute, taking a mixture of CO and air as the example[9]. The velocity of reaction between CO and air is known to increase

markedly in the presence of traces of hydrogen. It proved that mixtures of CO and air do not detonate but that the introduction of 1% hydrogen into such mixtures renders them capable of detonating.

A theory of detonation was developed in the institute which took reaction kinetics (i.e. the width of the reaction zone) into account[10]. In this theory, all the questions mentioned relating to detonation capacity and its limits found a semi-quantitative explanation, similar to the explanation of normal flame propagation limits.

A noteworthy phenomenon, the so-called «spin detonation» discovered by C. Campbell and W. A. Bone[11], was tested exhaustively in our institute[12]; these experiments led to the development of the first theory of this remarkable process.

We see now how comprehensive investigation of the chemical, physical, and mechanical peculiarities of a phenomenon and their reciprocal effects by a relatively small group of scientific collaborators in the Institute for Chemical Physics (A. V. Sagulin, O. M. Todes, D. A. Frank-Kamenetskii, Yu. B. Chariton, A. Ya. Apin, Ya. B. Zel'dovich, A. F. Belyaev, P. F. Pokhil, K. I. Shchelkin, A. S. Sokolik, O. I. Leipunskii) in conjunction with some foreign authorities (C. N. Hinshelwood, A. C. Egerton, B. Lewis, G. von Elbe, W. Jost, G. Damköhler, P. F. Laffitte, F. P. Bowden, T. von Kármán and many others) made it possible to lay the foundation of today's theory of combustion and explosion processes in gases and explosives.

There are virtually many respects in which this theory is not completely clear, and a vast field of work still confronts us all, but the main paths towards resolution of the problem are already marked.

In the years 1926–1927, we came in contact with another direction which constitutes a basic direction in chemical kinetics and which illustrates the intimate mechanism of chemical reactions. We established that not every auto-ignition is thermal in nature, but that there is a second, quite different kind of auto-ignition which we termed chain-ignition.

Thirty years have passed since Yu. B. Chariton and Z. Valta[13] discovered the lower limit of phosphorous ignition and since I concerned myself[14] (to a certain extent in conjunction with A. I. Shal'nikov) with research into the properties of this phenomenon, as well as since the first experiments at explaining this phenomenon theoretically by notions of branched chain reactions and their termination at the vessel walls (soon afterwards, this was proved in direct experiments by A. Trifonov[15]). The results obtained were so significant that the investigation of chain reactions, particularly

branched chain reactions, became the principal problem of the Institute for Chemical Physics for many years.

The branched chain surge can occur under isothermal conditions (in which the heat of reaction is fully dissipated), whereas in the thermal surge, the dissipation of the heat renders an explosion impossible. In the branched chain reaction, active particles, namely free radicals, are produced, the number of which grows quickly, due to the branching of the chain.

It is therefore enough for one single free radical to be produced (for example thermally) for the radicals to multiply, which leads to a rapid reaction of the chain ignition type.

The theory was examined particularly thoroughly in the experiments conducted by V. N. Kondrat'ev, E. I. Kondrat'eva[16] and L. I. Avramenko[17] who investigated the reaction of chain ignition in hydrogen–oxygen mixtures (close to the limit where the temperature of the flame does not exceed 700 to 800° C), and in the experiments of N. M. Emanuel[18], who was concerned with the slow reaction of hydrogen sulfide oxidation (the reaction took some minutes). In both cases, the presence of radicals (H, OH, SO) was observed directly, the number of H atoms rising up by 10% compared with the starting hydrogen, under certain conditions and during the oxidation of the hydrogen, whilst the number of SO radicals rose, during oxidation of the hydrogen sulphide by up to 40% as compared with the outset. At the end of the reaction, naturally, all the radicals had disappeared. The radicals disappear because they are trapped by the vessel walls, because they react with special admixtures or by virtue of recombination processes. This is when the chains break.

Where branched chain reactions are concerned, there are two possibilities: (1) the rate of branching exceeds that of termination, which results in very rapid development of the chain avalanche; (2) the rate of termination is greater than that of branching, so that the avalanche cannot develop and the reaction cannot even take place (if, as is usual, the rate of free radical formation is quite low); then, for example, phosphorous vapours may stay below the limit for days and in contact with oxygen without even a trace of reaction processes being observed. The transition from a completely inert state to a violent reaction (explosion) can easily be caused by for example increasing the pressure of the oxygen or the dimensions of the vessel, or by inserting tiny metal wires which violently absorb the free radicals, into the vessel or withdrawing such wires from the vessel, or by adding inert substances, etc. The transition from a practically entirely inert state to an en-

ergetic reaction occurs if one of these parameters varies within extremely close limits which, on some occasions, are virtually unmeasurable. Similar limit phenomena were observed by Sir Cyril Hinshelwood and collaborators[19] in the case of pressures which exceed a certain « second upper limit » (e.g. in the case of the reaction of water formation from oxygen and hydrogen). The second upper limit was explained on a basis of the ideas put forward by Sir Cyril Hinshelwood regarding chain termination in the volume in the case of threefold collisions and the concept of chain branching.

This meant that both our investigations and those conducted by Sir Cyril Hinshelwood[19] and his collaborators led to the establishment of a new idea of the reaction limit, as the result of each branched process which is accompanied by chain termination. These researches were conducted at the end of the twenties and start of the thirties.

When, at the end of the thirties, the physicists discovered branched nuclear reactions, similar limit phenomena were observed as with the chemical reactions, in spite of the difference which obtained in principle, because limit phenomena of this type characterize any branched chain reaction. With nuclear reactions, it is possible to observe with particular clarity the transition from the perfectly inert condition to an accelerated reaction or even explosion, following a change in such parameters as size and density, etc. (for example when control rods are introduced into or moved out of nuclear reactors).

From the field of the slow chemical reactions, we ought first to quote chain reactions with degenerate branching. These reactions are characterized in that their primary product is a molecular intermediate which enters relatively slowly into the reaction leading to the formation of free radicals. The existence of such reactions was proved even in the thirties, when it was established that the reactions of hydrocarbon oxidation are degenerately branched. The theory of « degenerate explosions », which we developed in 1931–1934[20] is today generally accepted.

As is shown by the work of A. Ya. Apin[21], not only the oxidation processes but also the decomposition of nitrogen chloride has the character of a branched chain reaction with an upper ignition limit.

Most other chemical gas reactions constitute unbranched chain reactions. With such reactions and also with the branched reactions, chain propagation takes place rapidly due to the formation of free radicals. Since however there are no branchings, there is no spontaneous development. Each chain is produced only by reason of the action of a spontaneously forming free

radical (e.g. under the dissociation of the starting substance due to thermal motion).

At this point, I would like to review the development of notions in the field of chemical processes. The problem of chemical transformations and the regularity observed thereby is probably one of the main problems of chemistry and chemical technology. That is why the development of the general theory of chemical reactions and reaction mechanisms is so important.

Actually, the significance of this question was acknowledged by chemists (particularly by those who were active in the field of organic chemistry) some considerable time ago, and they systematized their years of experience in the form of various reactivity rules which were often connected with the theory of structure. Unfortunately, these rules were in most cases only qualitative; they had many exceptions and were often dependent upon the test conditions. Nowadays, roughly a hundred years since the advent of the theory of structure, questions of reaction velocity are dealt with from an electronic and quantum-mechanical standpoint. There can be no doubt that great progress has been achieved in this field. Nonetheless, the theory still remains qualitative and often we have to make do by just substituting one certain terminology for another.

In the second half and particularly in the last quarter of the last century, chemists' attention was drawn to another aspect of chemical reactions which I should like to characterize as physical. From that time on, physical chemistry became acknowledged as an independent science which introduced thermodynamic and statistical methods of investigation into chemistry. And so chemical kinetics–the science of the velocity of chemical reactions–was created. The first successes in this field and in other fields of physical chemistry are linked with the names of J. H. van 't Hoff and S. Arrhenius.

Van 't Hoff and Arrhenius discovered some basic laws of chemical kinetics. In the view of Van 't Hoff, all chemical reactions ought to occur as simple mono- or bimolecular reactions if only their progress is not affected by perturbation influences; these latter might for example be caused by secondary reactions, the liberation of heat or catalytic effects.

With the exception of Van 't Hoff himself, who was greatly interested in perturbation influences of this type, most authorities confined themselves in the decades which followed to investigating the kinetics of such reactions as followed the simple laws. Since the number of such reactions is quite small, this limitation led to inhibited development of kinetics.

Nonetheless, it must at this point be remarked that, at the end of the last

and the beginning of the present centuries, a number of investigators were concerned also with complicated reactions in the field of conjugated oxidation, homogeneous catalysis and auto-catalysis in electrolytic solutions. I am referring to the work of Wi. Ostwald, N. A. Menshutkin, D. P. Konovalov, R. Luther, F. Haber, A. N. Bach, S. Engler, N. A. Shilov, V. Goldschmidt, J. N. Brønsted and others. Among these workers, there were many which drew attention to the significance of often unstable intermediates to the course of chemical reaction.

It was roughly about this time that Max Bodenstein started his scientific activities. In his investigations in the field of gas reactions, he concerned himself not so much with tracing simple reactions but was on the contrary interested in the course of complicated reactions (see for example his classic work on the reactions between hydrogen and bromine, 1907). As is well known, Bodenstein, in investigating photochemical reactions of chlorine and hydrogen, made in 1913 the outstanding discovery of the enormous quantum yield of this reaction which contradicted Einstein's law.

And so the idea of chain reactions was introduced into chemistry for the first time*. It is impossible to overestimate the importance of this work to the development of chemical kinetics as a whole.

A correct interpretation of the mechanism of the hydrogen–chlorine reaction was given some years later by W. Nernst, who assumed that the chlorine molecule decomposed under the action of light into two atoms which initiated a long chain of transformations:

$$\dot{\mathrm{Cl}} + \mathrm{H_2} \rightarrow \mathrm{HCl} + \dot{\mathrm{H}}$$
$$\dot{\mathrm{H}} + \mathrm{Cl_2} \rightarrow \mathrm{HCl} + \dot{\mathrm{Cl}}, \text{ etc.}$$

Enlarging on the findings of M. Bodenstein, J. A. Christiansen, in 1924, connected the inhibiting action caused by traces of a few active additives (inhibitors) on oxidation reactions in liquids, with the chain-nature of the reaction process and with the termination of the chain produced by these inhibitors.

In 1927, Bäckström proved the accuracy of this supposition and used this method to establish the chain mechanism of various reactions. For some reason, no adequate importance was attributed to this work. In reality, it is among the most important works in the history of chain reaction.

* A review of the history of the development of chain reactions up to 1934 is given in the book *Die Kettenreaktionen* (Chain reactions) by N. N. Semenov[20].

I would also like to mention the work of J. A. Christiansen and Kramers in 1923, when they applied the chain theory to thermal reactions, particularly monomolecular reactions. The truly interesting view formulated by these authors relating to pure energy chains could not subsequently be confirmed by experiment, so it was dropped. Nevertheless, it is possible that in individual radical reactions, energy factors play an important role; this is demonstrated particularly by the interpretation of the positive interaction of chains which we observed and by considerations of fast reactions in hot flames, as described recently in the literature[22].

And so we see that increasing interest was devoted to chain reactions in the twenties. Certainly, at that time, the number of chain reactions known was fairly small and such reactions seemed rather to constitute an exception.

In the years 1926–1928, both C. N. Hinshelwood[19] and we commenced investigations in the field of chain reactions. In 1934, in my book *Chain Reactions*[20], I had the following to say regarding the importance of these works:

« In 1927–1928, in Oxford, Leningrad and to a certain extent in Princeton, the chain theory was applied to investigate the reaction of such substances as are capable of igniting and exploding.

The most important feature resides in the fact that the theory was very closely linked with experiments which led to the discovery of new phenomena and also to the explanation of some long-known but hitherto incomprehensible phenomena. These works made it possible to draw up quantitative laws for chain reactions valid for a large group of phenomena and simultaneously made it possible to define the specific field of these phenomena. As the result of such works, increased interest was given to reactions of this kind, which, in 1930–1933, led to many new investigations being conducted. We would like to think that these works constitute the basis for a redevelopment of chemical kinetics. »

In the years 1929–1935, a large number of the most widely diverse experiments were conducted to explain and further develop the theory. Apart from our group of collaborators (V. N. Kondrat'ev, A. V. Sagulin, A. A. Koval'skii, Yu. N. Ryabinin, A. Trifonov, M. B. Neuman, V. R. Bursian and V. S. Sorokin, A. B. Nalbandyan, N. M. Chirkov, F. I. Dubovitskii) and the Hinshelwood group (H. W. Thompson, C. H. Gibson) various other researchers, e.g. B. H. L. Emeleus, R. G. W. Norrish, H. W. Melville, E. B. Ludlam, R. N. Pease, B. Lewis, G. von Elbe and many others concerned themselves with branched chain reactions.

The year 1931 saw the publication of works by F. Haber and R. Willstätter in which it was shown that ions of varying valence can readily form free radicals in some solutions. In the view of these authors, some important organic, particularly biochemical, reactions follow a chain mechanism.

In 1934, my book *Chemical Kinetics and Chain Reactions* appeared in which a general theory of branched and non-branched chain reactions was presented and in which it was shown, on the basis of experimental data, that such reactions formed by far the biggest part of gas reactions and are often to be encountered in chemical transformations in the liquid phase.

The accuracy of the supposition that polymerization processes also have a radical-chain nature was proved shortly afterwards experimentally by, among others, S. S. Medvedev[23]; F. O. Rice (USA)[24] and, later on, A.V. Frost (USSR)[25] proved the chain mechanism of cracking processes.

A substantial difference between the traditional theory of chemical reactions and the chain theory resides in the fact that the latter is not delimited by any perturbation influences but, on the contrary, sets out to provide general theoretical bases, with consideration to the courses of these influences. The laws of Van 't Hoff and Arrhenius still form the foundation of chemical kinetics but, in the majority of cases, they ought only to be used for elementary reactions.

The importance of chain reactions to technology is shown by the fact that most industrial processes, such as for example cracking, combustion, polymerization, slow oxidation, chlorination, are all typical chain reactions. The field of chain reactions and radical reactions has become a principal branch of modern theoretical chemistry.

At present, various laboratories both here and abroad (in this respect, I would like particularly to mention the successful work of the School of E. W. R. Steacie), are exhaustively investigating the mechanism of chain reactions; methods are being developed to form and examine free radicals. Moreover, the effect of the structure of the radicals on their chemical activity and various other questions appertaining thereto are being investigated.

I should now like to report rather more about the work of our institute which has interested me particularly during the last 5–6 years. Some of the work was commenced only a short while ago; I would therefore like to stress that some of the theoretical conclusions cannot as yet be regarded as final.

Of great interest is the problem of competition of chain reactions and ordinary molecular reactions which are not accompanied by the formation

of radicals. Chain propagation (a reaction between molecules and radicals) usually occur at a fairly high velocity, at least where the temperature is relatively high. The slowest stage is the initiation of a chain which is often combined with increased activation energy. For this reason, the reaction rate is in fact higher with artificial chain initiation (e.g. in the case of photochemical processes or by the action of additives) than for any molecular reaction. If no such artificial chain initiation occurs, then considerable significance must be attributed to the formation of free radicals by molecules participating in the reaction. The easiest way to form free radicals lies in the decomposition of the weakest bond of the molecule. The extensive investigations into elementary decomposition reaction carried out recently by M. Szwarc[26] and other authors (to a certain extent in our institute also) showed that, in some cases, the decomposition of molecules occurs more readily in radicals than in saturated fresh molecules. It follows from this that thermal decomposition of such substances proceeds by a radical-chain mechanism.

In other cases, though, the dissociation into radicals occurs more slowly than into molecules. An example of this is the decomposition of some organic chlorine and bromine derivatives of hydrocarbons. The competition between these two kinds of molecular decomposition depends upon their structure (C_3H_5Br[27] and C_3H_5Cl[28] for example split off one halogen atom primarily when they dissociate, whereas C_2H_5Br[29] and C_2H_5Cl[30] immediately split off hydrogen halide whilst simultaneously forming olefins; CH_3Br splits off one bromine atom[31] and, for chloroform, we proved immediate dissociation into hydrogen chloride and the biradical CCl_2[32]).

I would like to remark that, in some cases, even at a somewhat higher rate of decomposition into molecules, chain reaction can be decisive. This can be explained in that, due to the chain length, the chain reaction can occur more quickly than molecular reaction, even at a low rate of chain initiation.

The question of competition between a molecular and a chain mechanism has been the subject of several discussions, but it cannot yet be regarded as explained. This is partly due to the fact that chain reactions often follow simple kinetic laws and can therefore be similar to molecular reactions. Now, we also know of some reactions for which, under certain conditions, the molecular mechanism can be regarded as having been proved. This is particularly the case when the propagation of the chain is inhibited on account of the molecular structure.

Some interesting conclusions can be drawn from investigations into the

relationship between molecular structure and the mechanism of decomposition which were carried out in our institute and also independently by A. Maccoll[33] and others. For example, the decomposition of two bromides, namely *n*-propyl bromide and isopropyl bromide may be mentioned[34]. Whilst the first decomposes according to a chain arrangement, the second breaks down directly into molecules of hydrogen bromide and propylene. This can be explained as follows[35]:
in the case of *n*-propyl bromide, the following chain reaction occurs:

$$(0)\ C_3H_7Br \rightarrow\!\!\!\!\rightarrow \dot{C}_3H_7 + \dot{B}r$$
$$(1)\ \dot{B}r + CH_3CH_2CH_2Br \rightarrow\!\!\!\!\rightarrow HBr + CH_3\dot{C}HCH_2Br$$
$$(2)\ CH_3\dot{C}HCH_2Br \rightarrow\!\!\!\!\rightarrow CH_3CH{=}CH_2 + \dot{B}r \quad \text{etc.}$$

whilst, in the case of isopropyl bromide, the reaction

$$(1)\ \dot{B}r + (CH_3)_2\,CHBr \rightarrow\!\!\!\!\rightarrow HBr + (CH_3)_2\dot{C}Br$$

leads to formation of the radical $(CH_3)_2\dot{C}Br$, which cannot decompose further, and so inhibits the chain reaction.

Why are chain reactions so widespread?

It must be assumed that they are determined by some general fundamental chemical laws.

Firstly, the free radicals are chemically highly active particles and therefore react far more readily with molecules than molecules react to one another (in homolytic reactions).

Secondly, the free valence cannot disappear in the course of the reaction between a free radical and a molecule, which means that at least one product of reaction has the character of a free radical. This radical, for its part, again reacts with the nearest molecule, so that another free radical is formed, and in this way, a long chain of changes can take place.

In this respect, three cases are possible:
(1) During the course of the reaction between radical and molecule, a monoradical forms, which leads to the formation of an unbranched chain, e.g.

$$\dot{C}H_3 + CH_3CHO \rightarrow\!\!\!\!\rightarrow CH_4 + CH_3\dot{C}O$$
$$CH_3\dot{C}O \rightarrow\!\!\!\!\rightarrow \dot{C}H_3 + CO \quad \text{etc.}$$

(2) During the course of the reaction, instead of one free valence, three are formed, in other words three monoradicals of which each one in turn starts a chain. The result is the formation of a branched chain reaction, e.g.

$$\dot{H} + O_2 \rightarrow\!\!\!\!\rightarrow \dot{O}H + \dot{O}$$
$$\dot{O} + H_2 \rightarrow\!\!\!\!\rightarrow \dot{O}H + \dot{H}$$
$$\dot{O}H + H_2 \rightarrow\!\!\!\!\rightarrow H_2O + \dot{H}$$

which corresponds to the following overall process

$$\dot{H} + O_2 + 3H_2 \rightarrow\!\!\!\!\rightarrow 2H_2O + 3\dot{H}$$

(3) If the main chain of the reaction is unbranched but if, due to monomolecular breakdown of reaction products or if, in any other way free radicals and, consequently, new chains can form, then the characteristic features of branched chain reactions can be discerned in the reaction (autoacceleration, limiting phenomena). In this case, it is usual to speak of degenerate branchings. As an example, we might quote the formation of alkyl hydroperoxides:

$$\dot{R} + O_2 \rightarrow\!\!\!\!\rightarrow R\dot{O}_2$$
$$R\dot{O}_2 + RH \rightarrow\!\!\!\!\rightarrow ROOH + \dot{R}$$

Due to the small amount of decomposition energy in the bond O–O (30 to 40 kcal), the hydroperoxides decompose relatively easily and at the same time form free radicals:

$$ROOH \rightarrow\!\!\!\!\rightarrow R\dot{O} + \dot{O}H$$

which raises the chain number.

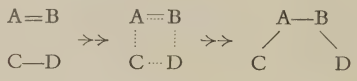
The high reaction capacity of free radicals is first and foremost an experimental fact and can to a certain extent also be explained by quantum mechanics. A calculation along these lines shows that, for the reaction A + BD $\rightarrow\!\!\!\!\rightarrow$ AB + D, namely in a linear arrangement of the atoms in the transitional state A... B... D..., the activation energy is usually not high (not more than 10 kcal). Where two molecules are acting

a rectangular transition complex is formed. The molecules CD draw close to the molecules AB in the direction perpendicular to the bond AB; in consequence, the activation energy levels here are high. If on the other hand, in the transitional state, the molecules assume a linear arrangement

$$A—B + C—D \rightarrow\!\!\rightarrow A\ldots B\ldots C\ldots D \rightarrow\!\!\rightarrow \dot{A} + B—C + \dot{D}$$

then, in the course of the reaction, two free atoms form and the reaction itself is markedly endothermic. Although, as we have been shown[35], the activation barrier is similarly low, as with the reaction between radical and molecule, the overall activation energy achieves quite high values due to the endothermic process.

With regard to the action of one atom or radical on a π-bond, an approach under a certain angle to the double bond ought probably to be the most advantageous. This activation stage of such reactions is similarly quite low (2–5 kcal). A fairly high activation energy in the case of a saturated molecule being added to a double bond



is probably connected with a necessary consumption of energy in the case of changes in configuration of the transition complex. In the case of an atom or radical being added, the configuration changes in the transition state are minimal, which explains the lowness of the activation barrier.

It must be mentioned that the magnitudes of activation energy determined experimentally for reactions between free radicals and molecules are quite various and fluctuate over a fairly wide range from 0 to 14 kcal. M. Polanyi showed[36] that, in a series of reactions between radicals and molecules of a uniform type, when the heat effect q rose, the activation energy ε_0 diminished steadily, rather following the law $\varepsilon_0 = A - \alpha q$. Once we had summarized most of the experimental data mentioned in the literature, we were able to show[35] that A and α, although they are not the same for different classes, are not greatly different. The empirical relationship $\varepsilon_0 = 11.5 - 0.25q$ gives for ε_0 values which do not differ by more than $\pm$ 2 kcal from values determined by experiment. At present, there are but few exceptions to this rule, mainly reactions with halogen, particularly with iodine.

With an approach of this kind, the activation barrier of the reaction and in consequence also the activity of the radicals and molecules can be deter-

mined as a difference in energy between the molecule bonds which are decomposing and those which are forming. A review of the experimental data for bond energy levels and for reaction capacities of radicals and molecules enables us to conclude that the activity of free radicals varies within fairly wide limits, even in the case of the free valence being linked with one and the same atom (for example C). This is due to the fact that, in the free radical, the p-electron is not paired, which naturally facilitates its interaction with the other part of the radical and leads to considerable conjugation energies in the radical. These energies depend naturally upon the structure of the radical. After the formation of a molecule during the course of the reaction, the electron is shown to be paired; it loses its «mobility» and is then only weakly joined with the other part of the molecule. Particularly marked is the conjugation of the free electron in the free radical in the case of its reaction with p-electrons of conjugated systems of π-compounds of the radical, which reduces the activity of such radicals. But even without such an interaction, substantiatedly easier action is possible through other causes (e.g. changes in configuration).

The facts mentioned make it possible to introduce the concept of relative activity of radicals which is not dependent upon which reaction of these radicals we use to determine them*. The activity of the radical NO_2 is 30 kcal less, of the allyl radical 21.5 kcal less, of the benzyl and CBr_3 radicals 17 kcal less and of the C_3H_7 radical 3 kcal less than that of the CH_3-radical (in other words the bonding energy R–X is less than for CH_3–X by the amounts shown).

Such approach relationships make it possible to estimate, for not too low temperatures, the rate constants for reactions between free radicals and molecules. This enables us to calculate (though highly approximate) the magnitude of velocity of elementary stages in complicated reactions, such as for example oxidation and cracking. Unfortunately, there is not as yet any clarity as to the steric factor in exchange reactions. According to E. Steacie[37], the steric factors for many reactions are supposed to be several magnitudes less than one. On the other hand, there are no theoretical considerations which allow us to estimate the magnitude of this coefficient; there are little experimental data available relative to steric factors and what there is, is in most cases contradictory.

* If we for example assume the activity of the radical $\dot{C}H_3$ to be nil, then the activity of the radical R is determined as a negative heat effect of the reaction $\dot{R} + \dot{C}H_3X \rightarrow\rightarrow RX + CH_3 - q$. As we have succeeded in showing, the heat effect, in line with the above-mentioned considerations, is almost independent of the nature of X.

Furthermore, there is still little information available about bond dissociation energies, required to determine q, which, among other things, is tied up with the limited possibilities of the methods used for determining the bonding energy; this also relates to the electron collision method. It is interesting to mention a new method of estimating the bonding energy and proton affinity of the molecule, which has been proposed in our institute by V. L. Tal'roze. This method is based on the fact established by Tal'roze[38] and then by D. P. Stevenson[58] that the activation energy of the elementary reactions of ions and molecules is very small.

From the field of complicated reactions, we have in recent times been devoting the greatest attention to the slow oxidation processes. It is particularly characteristic of this work that the kinetics of the accumulation of all the main products of reaction should be undergoing investigation and balance be established in the course of reaction. In the gas phase, the thermal oxidation of propylene and propane has been thoroughly investigated. A. B. Nalbandyan[39] investigated the photochemical oxidation of propane and methane. The results gained by V. Ya. Shtern[40] on the kinetics of propane and propylene oxidation make it possible to suggest a reaction mechanism which, in turn, makes it possible to describe the kinetics of the accumulation of most products of oxidation and by-products of cracking. This reaction mechanism is based on the assumption of two competing transformation possibilities of the radical RO_2 which forms as an intermediate product – a bimolecular transformation with hydrogen splitting off from the hydrocarbon molecule and the formation of hydroperoxide and a monomolecular transformation with isomerization of RO_2, subsequent decomposition of the carbon skeleton and formation of carbonyl compounds (without peroxide forming). The degenerate branching is, from experimental data, probably the result of an oxidation of acetaldehyde.

In the liquid phase, N. M. Emanuel[41] exhaustively investigated the oxidation of cyclohexane and *n*-decane, determining the sequence of the formation of stable reaction products and, probably for the first time, he showed for paraffins and cycloparaffins that all products of reaction form due to the transformation of a primary intermediate material – hydroperoxide. It is interesting to note that the phenomena are somewhat different if the reaction is carried out in a metal apparatus. According to provisional results regarding oxidation of cyclohexane in a steel apparatus, the intermediate stage, with the formation of hydroperoxide, is avoided during formation of the main mass of reaction products. Since, as we know, the alkyl peroxide formation

is a homogeneous chain reaction, such a change in the mechanism shows that the walls of the reaction vessel influence propagation of the chain.

Of great importance to the work was the kinetic method developed by M. B. Neuman[42] using active isotopes; this method made it possible to measure the rate of formation and disappearance of stable intermediates and hence to determine the sequence in which they are formed.

During the course of recent years, our institute commenced investigations into the mechanics of the thermal decomposition of hydrocarbons. Comprehensive attention to literature data regarding cracking processes of olefins led V. V. Voevodskii[43] to conclude that these processes obviously did not, as was hitherto assumed, constitute radical but rather more chain reactions. The mechanism developed by the author is based on the assumption of a hydrogen exchange between free radicals and olefin molecules, e.g. for a radical obtained from olefin C_nH_{2n}

$$\dot{C}_nH_{2n-1} + C_nH_{2n} \rightarrow\!\!\!\!\rightarrow C_nH_{2n-2} + \dot{C}_nH_{2n+1}$$

On a basis of this assumption, the author was able to show the composition of the cracking products for a number of olefins.

Of the utmost practical interest is the initiation of a chain, i.e. to the formation of primary free radicals and atoms. To this end, the effect of light, electrical discharge, addition of substances which decompose easily in radicals and, particularly of late, radiation has been used. I would like here to deal mainly with such chain initiation processes as are, in theory, still not adequately clear although they are directly linked with questions of chemical kinetics.

It can be assumed that free radicals often form under the action of two molecules[35] (and not during dissociation of molecules into radicals). As already mentioned, quantum-mechanical calculations show that such reactions can take place with a favourable molecular arrangement yet even without any great activation barrier. This conclusion can also be proved experimentally. It is known that the reaction of two radicals is not by any means restricted always to valence saturation, with a molecule being formed from both particles. It is quite often the case that disproportionation processes occur, as for example

$$C_2H_5 + C_2H_5 \rightarrow\!\!\!\!\rightarrow C_2H_4 + C_2H_6$$

As can be proved by experiment, such reactions exhibit no activation energy

at all, or if they do, then only a very slight one. From this, taking general thermodynamic considerations into account, it follows that the activation energy of the reciprocal reaction of the formation of free radicals, where two molecules are involved, e.g. $C_2H_4 + C_4H_6$, must be equal to the heat effect of this process. In this case, the energy consumption is fairly high (62 kcal), yet not as high as in the case of the formation of radicals upon dissociation $C_2H_6 \rightarrow\!\!\rightarrow 2CH_3$ (83 kcal) or even in the case of the dissociation $C_2H_6 \rightarrow\!\!\rightarrow C_2H_5 + H$ (98 kcal).

The chain oxidation of liquid substances is often characterized by a fairly high initial velocity, i.e. by a high rate of initiation. If the above consideration are extended also to such systems, then the reaction must be described in the following way: $RH + O_2 \rightarrow\!\!\rightarrow R + HO_2 - q$. If RH is aldehyde, e.g. acetaldehyde, the bonding energy is no more than 80 kcal. The energy of the reaction $H + O_2 \rightarrow\!\!\rightarrow HO_2$ was determined fairly accurately and amounts to 45–47 kcal. The heat effect q is therefore some 35 kcal and is about the same magnitude as the decomposition energy of peroxides. The results established in recent years as regards oxidation of gaseous acetaldehyde and ethyl linoleate have shown that the chain initiation is probably decided by the bimolecular reaction of the oxidizing substance with oxygen. We know too that the addition of oxygen accelerates cracking processes and other organic reactions (e.g. hydrobromation of olefins). In all these cases, the action of the oxygen can be explained by the aforesaid chain initiation: $RH + O_2 \rightarrow\!\!\rightarrow R + HO_2$. The energy consumed in this reaction is about 30 kcal, which means that it is of the same magnitude as in the decomposition of peroxides (the bonding energy C—H in the CH_3 group of propylene is 77 kcal).

Many hydrocarbon oxidation processes have the nature of chain reactions with degenerate branchings, which often leads to considerable induction periods. In the liquid phase (e.g. in the oxidation of paraffin), such induction periods often extend to some hundreds of hours. As we know the introduction of small quantities of nitrogen oxides reduces in the gas phase the induction period of oxidation of hydrocarbons at high temperatures; the cause lies in the accelerated formation of radicals by the effect of nitrogen oxides on the hydrocarbon molecule. N. M. Emanuel and his collaborators[44] recently showed that nitrogen oxides have a similar action also during oxidation in the liquid phase, at a relatively low temperature. In order to commence the reaction, it is sufficient to pass air with a low NO_2 content through liquid paraffin for a short time. After such a reaction has been started, continuance of the reaction is ensured by the peroxides which form. At this stage, pure

air can be passed through the paraffin; admixture of nitrogen oxide has even a rather inhibiting effect on the rate of reaction (probably due to decomposition of the peroxides). These phenomena have been proved for both liquid and liquefied paraffins.

According to observations by A.V.Topchiev[45], methane can be nitrated and chlorinated easily under the simultaneous action of NO_2 and Cl_2; chlorination without NO_2 or nitration without Cl_2 takes place far more slowly. This can probably be explained by the formation of NOCl which for its part generates chlorine atoms.

A noteworthy phenomenon of chain initiation was discovered recently by N.M.Emanuel[46] in his work on the oxidation of propane in acetone at 180 to 200° C in the presence of HBr. He established that, in the initial state of propane oxidation, a reaction takes place during the few seconds following introduction of the reaction components, during the course of which only infinitesimal quantities of propane are consumed. But actually during the course of this reaction, such active particles form as determine the main stage of the process, which may extend over several minutes. It is interesting to note that the reaction product acetone inhibits the reaction and that, if there is an adequate initial concentration of acetone, the reaction does not take place at all. If, on the other hand, acetone is introduced into the reaction zone after completion of the first stage (fractions of minutes after its commencement), this no longer has any effect at all on the course of the reaction. We see, therefore, that acetone acts only on the first, initiating stage of the reaction. It has not yet been possible to ascertain the chemical mechanism of this process. Possibly it may be a branched chain reaction between HBr, propane and oxygen, during the course of which active intermediates are formed which, in turn, decompose to form free radicals, so initiating the chain reaction of the main stage of propane oxidation.

Although as yet without foundation, the possibility of chain initiation by the solid walls of the vessels opens up vast perspectives.

As a result of the work of A.A.Koval'skii[47] (who worked out a method of differential calorimetry), it may be considered as firmly established that for some heterogeneous catalysts – e.g. bauxite catalysts in the industrial recovery of sulphur from CO and SO_2 – the effect is confined to the initiation of volume reactions on the surface, whereas the actual reaction occurs in up to 96% in the gas phase. Using differential calorimetry, A.M.Markevich[48] demonstrated, some years ago, that the chains of the dark reaction $H_2 + Cl_2$ start on the surface of the glass. A.M. Chaikin[49] came to a similar conclu-

sion for the dark reaction of dichloroethane formation during the chlorination of ethylene. The same author furthermore showed, for the first time and with complete clarity, that the direct addition of chlorine to ethylene does not occur in the gas phase and that the reaction always is of a chain nature, chlorine atoms participating.

As already mentioned earlier, we succeeded in the twenties in proving the possibility of chain termination on solid surfaces. Subsequent research into this phenomenon, both in our country and abroad, showed that atoms and free radicals are almost always chemosorbed by solid surfaces. The activation energy of the chemosorption process depends upon the nature of the surface but it is always fairly low (less than 10 kcal).

Passing on to the theory of reaction initiation by the vessel walls, I would like first of all to establish that the fact of free radicals being trapped by the walls, as has been unequivocally established for the majority of chain reactions, leads, on thermodynamic grounds, to the reverse reaction of radical formation also taking place at the walls.

The initiation of chain reactions by the vessel walls has also been established in the decomposition of alkyl chlorides. Even earlier, it was known that, in clean, untreated vessels, this reaction takes place considerably more quickly (some magnitudes more quickly) than in vessels the walls of which have become coated during the course of repeated reaction processes. This phenomenon cannot be explained as heterogeneous catalysis on clean surfaces. Tests carried out recently in our institute with varying proportions of surface to volume, and also tests using differential calorimetry, show fairly clearly that, in many cases, the reaction is virtually homogeneous both in clean as well as in treated vessels. These facts can only be explained by assuming that the chain reaction is initiated on the surface. Obviously, this process takes place easily on clean surfaces and is made markedly more difficult on treated surfaces. Some researchers (Barton, Howlett[50] and others) investigated the extent to which the decomposition reaction in treated vessels is inhibited by the addition of propylene, *n*-hexane, and other inhibitors. In all cases where an inhibiting effect was observed, the velocity of reaction changed by 3 to 4 times in the presence of additives. Recently, we undertook an investigation into the action of propylene in clean vessels. It turned out that, in this case, inhibition is considerably more intense (20 times more marked). This difference in itself makes the general idea of the retarding action of propylene being connected with a chain termination in the gas phase, somewhat doubtful.

An exhaustive investigation of this question using differential calorimetry would appear to point to the fact that, in any case, the main proportion of inhibition (80 to 90%) is not connected with chain termination in the gas phase but with the suppression of chain formation at the walls, due to adsorption of propylene.

These results can be considered as a certain confirmation of the hypothesis of V. V. Voevodskii[51] regarding the action of NO admixtures on hydrocarbon cracking. In accordance with the comments of F. O. Rice and K. F. Herzfeld[52], Voevodskii assumes that the hydrocarbon chains form and break at the walls in the cracking process too. In his opinion, the nitrogen oxide inhibits the initiating action of the walls. These experiments to find a new solution to this question are connected with the fact that the acknowledged opinion of Sir Cyril Hinshelwood[53] on the nature of inhibitions in the cracking reaction, although it does offer some advantages, can only with difficulty explain certain test results. For example, some authorities[54] have shown that, in the case of reactions in the presence of molecular deuterium or stable deuterium compounds, the deuterium content of the reaction products is roughly the same in non-inhibited as in reactions inhibited by NO.

We set ourselves the task of investigating the mechanism of the formation of free radicals on solid surfaces. We consider it probable that, in solid bodies and particularly on their surface, there are free valences[35]. These can be considered in a similar light to the free radicals and therefore, their action on the molecule emerging from the gas phase has a low activation energy. The molecule decomposes: one of the radicals saturates the surface valence, becomes chemosorbed, and the other passes over into the gas phase.

The trapping of the free radical on the wall occurs by its chemisorption, but one or other bond in the solid is ruptured and at the same time, a new free valence forms on the surface. For this reason, these processes do not change the total number of free valences (neither on the wall nor in the volume); free valences are destroyed only as the result of their interaction.

Free valences can probably be created by the solid bodies being especially prepared. At high temperatures, they may occur spontaneously due to thermal movement. Also, the possibility of their formation through the release of energy in chemical reaction processes on the surface is not excluded, for example:

$$H_2 + Cl_2 + \text{wall} \rightarrow\!\!\rightarrow (H)_{ads.} + HCl + \dot{Cl}$$

$$(H)_{ads.} + Cl_2 \rightarrow\!\!\rightarrow HCl + \dot{Cl} + \text{wall}$$

With such a formation of two chlorine atoms, 15 kcal instead of 57 kcal are consumed in dissociation of the chlorine molecule in the gas phase.

The ideas indicated regarding the mechanism of the share which the wall has in initiating and terminating chains as yet remain very hypothetical. We have endeavoured to connect them with the properties of the semiconductive solid bodies[55]. The qualities of the current carriers in the semiconductors – the free electrons and the pores – are namely very similar to the properties of the free valences on the surface, as postulated by us, both having as they do the characteristic quality of the free radicals – they have an odd number of electrons. Taking quite a rough approximation, the electron and the pore in the semiconductor lattice (e.g. ZnO) can be equated to the ions of the radical ($\dot{Z}n^{+}$ and $\dot{O}^{-}$).

In line with this analogy, we assumed in 1953 that the free valences on the wall did not alone contribute to ejecting the radicals into the gas phase, but also played a substantial role in the mechanism of heterogeneous catalysis in the redox processes. Some schemes have been suggested which describe in a fully satisfactory way the progress of some complicated catalytic reactions, for example the chain scheme of the synthetic process of $CO + H_2$.

At the moment, there are still no direct test results which confirm the radical mechanism or the ion-radical mechanism of the redox processes. However, there are quite a number of observations which provide indirect confirmation. The fact must be emphasized particularly that the greatest number of redox catalysts are semiconductors and metals[56]. On the other hand, quite a lot of data has been collected relative to comparative investigations into the electrical conductivity and catalytic properties of semiconductors. All these data point to the fact that these two categories of properties are closely linked with each other. A particularly clear indication of this is the effect produced by addition of small quantities of ions of a different valence into the semiconductor lattice (e.g. Li^{+} and Cr^{+++} into the lattice of $Zn^{++}\,O^{--}$, $Ni^{++}\,O^{--}$, etc.); when this happens, the electrical conductivity undergoes a sharp change, just like the catalytic activity, becoming either greater or smaller. Any admixture which influences the electrical conductivity also always causes changes in the catalytic activity. Sometimes, these changes occur in one and the same direction, but sometimes in an opposite direction, depending upon the type of reaction, the properties of the admixtures, the p- or n-character of the semiconductor and upon the temperatures. Although as yet we have no detailed theory at our disposal which is capable of describing all these data, it can probably be asserted that such a theory

must be founded on an exact picture of free surface radicals and such a theory would enable us, working from a uniform standpoint, to combine both the electrical and the catalytic peculiarities of the process.

We would like to mention here that, also with the acid and base catalysts, as N. M. Chirkov has shown, a connection exists between the electrical conductivity and the catalytic properties, but this connection is of quite a different kind. In 1947, we found[57] that in the presence of small quantities of hydrochloride gas and water vapours, surface plates of mica, quartz and glass acquired marked properties of acid–base catalysts. The esterification reaction and decomposition of paraldehydes were thoroughly investigated, and it became manifest that the surface electrical conductivity changes in parallel with the catalytic activity. Further investigations showed us that the reaction in the adsorption layer (which contains either one or a few layers of molecules) takes place largely just as in the acidified liquids.

The main outcome of many years' work by various chemical-kinetic schools is the certainty that a large number of chemical reactions in the gas phase and also by far the majority in the liquid and solid phase, as well as the heterogeneous catalytic reactions on solid surfaces, are aided by particularly labile intermediate forms which are produced during the course of the process. These labile intermediate forms have a considerably greater reactivity than even the least stable molecules with saturated valence bonds.

The development of theoretical and experimental processes of investigating these labile intermediate forms must be regarded as the most important task for the immediate future. We must clearly tie up the properties of these labile intermediate forms with their chemical structure or the structure of the molecules from which these labile forms are produced. We must clarify in detail how the process itself can generate such labile intermediate forms and so secure its own auto-development.

I see the value of the chain theory first of all in that it has succeeded, taking examples from gas reactions, in developing general notions relative to the simplest labile forms corresponding to these reactions – the free atoms and radicals. In a few cases, it has been possible to show that the doctrine relating to chain reactions constitutes an applicable theory which makes it possible quantitatively to foretell the astonishing wealth and manifold courses of the reaction processes which are characteristic even of such simplest chain processes as the formation of water from hydrogen and oxygen.

The chain theory makes it possible to come nearer to the solution of a

main problem in theoretical chemistry – the connection between reactivity and the structure of the particles entering into the reaction.

For chain processes, the reaction of free radicals with the molecules is characteristic (the reaction of chain propagation). Such reactions belong to the simplest because here only one bond is disrupted and one bond is generated afresh. Therefore, the investigation of these simple reactions offers the best opportunity for breaking fresh ground in the development of the theory of reactivity and it can be applied subsequently to other types of reaction also.

It is one of the main tasks of chemistry to create a possibility of rationally regulating the rate and direction of a chemical change. Unfortunately, chemistry and the chemicals industry are in this respect lagging behind radio technology, electronics, atomic engineering, etc., where technology is based far more on theoretical anticipation and calculation. The chain reaction theory shows the first possibilities in this respect also.

That the chain reaction includes the stages of initiation of the chain and its termination, which are greatly dependent upon the average condition and upon external influences, opens up the possibility of regulating the rate and sometimes even the yield of the process. The knowledge of the reaction of chain propagation often makes it possible to control the process in the desired direction.

Accordingly, the chain theory provides fresh prospects of perfecting established industrial processes and others which are new and in the development stage, and is already to a certain extent being applied; this is particularly true with polymerization processes.

One of the next tasks is the application of the notion of chain theory to the development of new technology of direct oxidation processes and hydrocarbon cracking. I would like further to add that, for practical purposes of chemical technology, radioactive radiation probably offers very good prospects where chain reactions are concerned.

Certainly, chemical transformation processes are not limited purely to radical chain reactions which occur with the help of free radicals; a vast number develop with the help of quite different labile forms.

I am firmly convinced that it is entirely necessary to extend the research into the mechanism of various types of chemical reactions. It is hardly possible to enrich chemical technology to any extent without this knowledge or even to achieve decisive success in biology.

Naturally, there are considerable obstacles in the way. The chemical pro-

cess is the basic phenomenon which makes chemistry different from physics and makes the former a more complicated science. The development of the theory of a chemical process is also considerably more difficult than the development of the theory of chemical structure.

More than anywhere, the mutual endeavours of learned men in every country are needed in the resolution of this most important problem, in order to explain the secrets of chemical and biological processes, to the benefit of peaceful development and the well-being of mankind.

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Biography

Nikolai Nikolaevič Semenov was born in Saratov on April 3, 1896. He graduated from Petrograd University in 1917 and in 1920 he took charge of the electron phenomena laboratory of the Leningrad Physico-Technical Institute. He lectured at the Polytechnical Institute and was appointed Professor in 1928. In 1931, he became Director of the Institute of Chemical Physics of the U.S.S.R. Academy of Sciences (which has moved to Moscow in 1943); from 1944 he has been a Professor at the Moscow State University.

Semenov's outstanding work on the mechanism of chemical transformation includes an exhaustive analysis of the application of the chain theory to varied reactions and, more especially, to combustion processes. He proposed a theory of degenerate branching which led to a better understanding of the phenomena associated with the induction periods of oxidation processes. Semenov has made valuable contributions to the field of molecular physics; he has also carried out investigations on electron phenomena, dielectric breakdown and the propagation of explosive waves.

Semenov has written two important books concerned with his work. *Chemical Kinetics and Chain Reactions* was published in 1934 with an English edition in 1935. It was the first book in the U.S.S.R. to develop a detailed theory of unbranched and branched chain reactions in chemistry. *Some Problems of Chemical Kinetics and Reactivity*, first published in 1954, was revised in 1958; there are also English, American, German, and Chinese editions.

He became a Corresponding Member of the U.S.S.R. Academy of Sciences in 1929 and Academician in 1932: he was awarded five Orders of Lenin and the Order of Red Banner of Labour. He is a member of the Chemical Society (London), Foreign Member of the Royal Society, and foreign member of the American, Indian, German, and Hungarian Academies of Sciences. He also holds Honorary Doctorate degrees of Oxford and Brussels Universities, and since 1960 he has been Chairman of the All-Union Society for Propagation of Political and Scientific Knowledge.

He married Natalya Nikolaevna Semenova; they have one son and one daughter.

Chemistry 1957

Sir ALEXANDER ROBERTUS TODD

«for his work on nucleotides and nucleotide coenzymes»

Chemistry 1957

Presentation Speech by Professor A. Fredga, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Your Royal Highnesses, Ladies and Gentlemen.

Nucleotides and nucleotide coenzymes are words that may seem strange and abstruse, but these compounds are of great importance to all of us. We have such substances everywhere in our bodies and they regulate many of the processes of life. The term is derived from nucleus, which here refers to cell-kernels or nuclei. The Nobel Prize of this year has nothing to do with atomic nuclei, nuclear fission or hydrogen bombs. Nucleotides are regularly found in the chromosomes of the cell-kernels, where they are connected with the units of heredity, but also in the cell plasma. In combination with proteins they constitute the virus molecules and many coenzymes are nucleotides of low molecular weight but with a special structure. Thus they are a group of substances of very great biological importance, perhaps the most important of all.

The nucleotides have been known for nearly 90 years and they have been frequently studied by both chemists and biologists, but for a long time they were, from the chemist's point of view, an underdeveloped field of research. The difficulties were too great. Gradually it was established that they are built up of three different kinds of « building-stones » of quite different chemical character: phosphoric acid, a sugar, and a heterocyclic base containing nitrogen. I cannot express it more popularly, except to mention that these bases are compounds related to caffeine. Two different sugars are found, ribose and desoxy-ribose, and about half a dozen different bases. The simple building-stones may then be combined in hundreds or thousands to form macromolecules, the nucleic acids.

It is, however, not enough to know the building-stones; we must also know how they are connected to each other. The building-plan, the pattern or whatever you prefer to call it, must be very essential for the behaviour of the macromolecule in chemical and biological processes. The sugars and the heterocyclic bases are both somewhat complicated molecules, which may be connected to each other in several different ways, and finally it must be established how the phosphoric acid is bound. The task is very difficult; the

combination of three quite different kinds of building-stones in one macromolecule gives it a very special character and neither the traditional methods of organic chemistry, nor those of inorganic chemistry are directly applicable. It is, however, pre-eminently a task for an organic chemist, and for more than ten years Sir Alexander Todd has held a leading position in this field.

Some idea of the building-plan may be obtained by examining the products formed by partial degradation of the macromolecule into small fragments containing a limited number of building-stones. Conclusive evidence can, however, only be obtained by synthetic methods, by building up possible combinations of sugars and bases – with or without phosphoric acid – and comparing them with the degradation products. It is of course imperative to use such methods that the structure of the synthetic products is irrefutable.

The work has been very comprehensive, and many special methods have been evolved, but it is hardly possible to give a non-chemist a clear idea of the brilliant experimental work accomplished. Perhaps I should specially mention the methods for introducing phosphoric acid, the phosphorylation. In recent years, the fundamental role of phosphoric acid in the biochemical processes has become more and more evident, and the new phosphorylation methods – now approximating to those used in the biosynthetic procedures – are also of interest outside the special domain of nucleotide chemistry.

The building-plan of the nucleic acids is now established, at least in its outlines. We have a long chain, where the links are alternately sugar and phosphoric acid, and to each sugar molecule is attached a heterocyclic base as a small pendant. Thus there is an equal number of acid and basic groups. The different building-stones are always connected according to the same pattern and the difference between various nucleic acids must therefore be due to the kinds of bases and their relative arrangement. The number of different types is small – in a certain chain usually only four different bases occur – but in a macromolecule with thousands of appendant base molecules the number of possible combinations must be very great. We are familiar with the coding potentialities of the Morse alphabet, which has only two symbols, dots and dashes.

Through Sir Alexander's work a solid foundation is laid for the future development in this field. Starting from this work, other scientists have advanced very fascinating theories as to the arrangement of the chains; it seems that they may be coiled up as a helix with the bases inside. This model can perhaps explain how a nucleic acid chain can bring about the formation of

another similar chain or even of a protein. We are here approaching very fundamental biological questions.

The synthetic methods have also been successfully applied to the preparation of low-molecular nucleotide coenzymes, for instance the cozymase, which plays a part in alcoholic fermentation and other biochemical processes. The ways are now open for synthetic preparation of the different types occurring in nature. It is also possible to synthesize coenzymes with slightly modified structure and study the effect of these modifications on the activity, and hence gain better insight into the mode of action of the enzymes.

Sir Alexander Todd. Some fifteen years ago you started your work in nucleotide chemistry. You saw the great importance of this topic and you did not underrate the difficulties. Today the chemical structure of these compounds is established – in any case in its outlines – and a solid foundation is laid for future work by biochemists and biologists. Results of utmost interest have already been reported and others will follow.

An organic chemist is perhaps most impressed by your studies in phosphorylation. We know today that phosphoric acid is engaged in most biochemical processes, but we know rather little of how it works. Some years ago, you expressed the opinion that the methods used by the living organism owe their air of magic largely to our lack of knowledge of the simple chemistry of the esters of phosphoric acids. You have learned to handle these esters with amazing skill, and I am sure that in due time the air of magic will disperse.

In recognition of your services to chemistry and to natural science as a whole, the Royal Swedish Academy of Sciences has decided to bestow upon you the Nobel Prize for Chemistry for your work on nucleotides and nucleotide coenzymes. To me has been granted the privilege of conveying to you the most hearty congratulations of the Academy, and of inviting you to receive your prize from the hands of His Majesty the King.

Alexander R. Todd

Synthesis in the study of nucleotides

Nobel Lecture, December 11, 1957

I am deeply conscious of the very great honour which the Academy has done me by its recognition of my work in the field of nucleotides and nucleotide coenzymes, and it is in these circumstances a very special privilege to review this work before its members. And yet it is not an easy matter to review in the compass of one lecture a group of researches which have been my major interest over a period of some fifteen years, and to steer a safe course between the Scylla of generalities and the Charybdis of excessive detail. Nor is the content of these researches readily described in other than a highly technical way. For I am an organic chemist, albeit one who adheres to the definition of organic chemistry given by the great Swedish chemist Berzelius, namely, the chemistry of substances found in living matter, and my science is one of the more abstruse insofar as it rests on concepts and employs a jargon neither of which is a part of everyday experience. Nevertheless, organic chemistry deals with matters of truly vital importance and in some of its aspects with which I myself have been particularly concerned it may prove to hold the keys to Life itself.

In retrospect, the path which led me to the nucleotides and to the methods of study which I employ is relatively clear. After my undergraduate work in Glasgow, I had my first introduction to natural product chemistry in Frankfurt where I worked for two years with the late Professor Borsche on bile acids. Thereafter at Oxford I had the privilege of working on the anthocyanins and other natural colouring matters with Robert Robinson. It was from him that I learnt that synthesis is not merely a means of confirming a structure arrived at by degradative studies. Synthesis and degradation are complementary tools in structural work, and, properly used, synthesis can serve not only in the elucidation of structure, but can in many instances open up wider vistas regarding the significance and function of biologically important compounds. This view of synthesis in organic chemistry I have held ever since and it has played a major role in determining the pattern of all my subsequent researches.

In 1934, at the invitation of the late Professor Barger, I went to Edinburgh

to take up the structural investigation of the anti-beriberi factor, vitamin B_1. It was only natural that, following the synthesis of the vitamin with Bergel, I became interested in the specificity of vitamins and the reasons for their importance to living organisms. It was a fortunate circumstance that about this time biochemical studies had begun to reveal that the vitamins of the B group owe their importance to the fact that they are converted in the organism to more complex derivatives which function as coenzymes in metabolic systems. In these studies Swedish workers played a prominent part and some aspects of them were discussed by Theorell in his Nobel Lecture in 1955; coenzymes related to the B vitamins include *inter alia* flavin-adenine-dinucleotide (FAD) and cozymase or diphosphopyridine nucleotide (DPN). It seemed to me that, if the vitamins functioned in the form of nucleotide coenzymes, then it would be necessary to study the chemistry and mode of action of the latter if any progress were to be made in understanding vitamin specificity. But to do this meant in the first instance an attack on the chemistry of the nucleotides, a group of compounds which had been rather neglected by most chemists and about which we were consequently rather ill-informed.

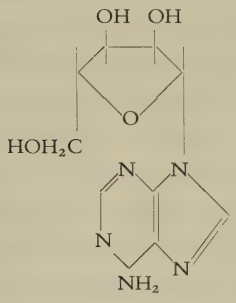
The term nucleotide requires definition, for, like many other terms, it is now used in a much broader sense than when it was first introduced. Originally it was applied only to the phosphate esters of certain *N*-glycosides of purine and pyrimidine bases (the nucleosides) obtained on hydrolysing nucleic acids. To-day it is applied generally and rather loosely to phosphates of *N*-glycosides of heterocyclic bases, and it includes not merely the simple nucleotides of the original definition but also the nucleic acids (polynucleotides) and such substances as nicotinamide nucleotide (5′-phosphate of the quaternary *N*-ribofuranosylnicotinamide), and adenosine triphosphate (ATP). The nucleotide coenzymes are, in general, characterized by the presence in them of at least one simple nucleotide residue and although derivatives of riboflavin phosphate (FMN) are not glycosidic in nature they are commonly listed among the nucleotides because of their close similarity to, and association with, true nucleotides.

In 1939 when I first began experiments in this field the fundamental substances of the group – the nucleosides obtained by hydrolysing nucleic acids – had long been known and had been the subject of study by various workers. The early studies of Fischer had been followed by those of a few other investigators among whom one thinks particularly of Levene. As a result it was established that the 4 nucleosides derived from ribonucleic acids were N-D-ribosides of adenine, guanine, uracil and cytosine respectively, but the

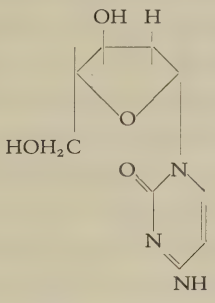
size of the lactol ring in the sugar residue and the configuration at the glycosidic linkage were unknown, while the point of attachment of the sugar residue in the purine nucleosides was still in dispute, although the spectroscopic evidence of Gulland and Holiday indicated with a high degree of probability that it was $N_{(9)}$. Of the nucleosides from deoxyribonucleic acids, all that was known with any certainty was that they were 2-deoxy-D-ribosides of the bases adenine, guanine, thymine and cytosine and it was assumed that they were structurally analogous to the ribonucleosides. The chemistry of the nucleotides – the phosphates of the nucleosides – was in a correspondingly primitive state. It may well be asked why the chemistry of these groups of compounds was not further advanced, particularly since we recognize today that they occupy a central place in the chemistry of the living cell. True, their full significance was for long unrecognized and emerged only slowly as biochemical research got into its stride but I think a more important reason is to be found in the physical properties of compounds of the nucleotide group. As water-soluble polar compounds with no proper melting points they were extremely difficult to handle by the classical techniques of organic chemistry, and were accordingly very discouraging substances to early workers. It is surely no accident that the major advances in the field have coincided with the appearance of new experimental techniques such as paper and ion-exchange chromatography, paper electrophoresis, and countercurrent distribution, peculiarly appropriate to compounds of this group. Without them and the availability of convenient and precise spectroscopic methods, I doubt whether our work would have been possible.

I decided that we should seek to clarify the nucleotide field beginning with the simplest units – the nucleosides. To do so we applied primarily the method of synthesis since the amount of preliminary information available from earlier work had at least given sufficient indication of the nature of the nucleosides to make such an attack appropriate. This phase of our work, although providing, I believe, an interesting example of the power of synthetic methods in structural work, would take an entire lecture to describe in itself and I shall not, therefore, discuss it here. Suffice to say that this work led to the rigid establishment of the structure of the individual ribonucleosides as the 9-β-D-ribofuranosides of adenine and guanine and the 3-β-D-ribofuranosides of uracil and cytosine and to the total synthesis of all of them. The deoxyribonucleosides were similarly shown to be 9-β-2-deoxy-D-ribofuranosides in the case of the purine, and 3-β-2-deoxy-D-ribofuranosides in the pyrimidine members. The difficulty of obtaining and of handling deriva-

tives of 2-deoxy-D-ribose has hampered synthesis of the natural deoxyribonucleosides, but deoxyuridine has recently been synthesized and it is likely that synthesis of the others will shortly follow. For reference, the structural formulae of two typical nucleosides – the ribonucleoside adenosine (I) and the deoxyribonucleoside deoxycytidine (II) are given below.



(I)



(II)

The simple nucleotides are phosphates of the nucleosides, the phosphate residue being attached to one or other of the hydroxyls in the sugar portion of the molecule. Phosphorylation of the nucleosides was thus a second essential phase in our studies. Although organic phosphates and polyphosphates are of widespread occurrence in living matter, relatively little attention had been paid in the past to their synthesis and still less to their chemical behaviour. True, a number of organic phosphates had been prepared, usually by rather crude procedures not well suited to use with sensitive molecules, and we found it necessary to undertake a general study of phosphorylation in all its aspects so as to make available methods which might be satisfactory in dealing with the rather wide range of delicate structural features to be encountered in the nucleotide and nucleotide coenzyme field.

The most widely employed phosphorylating agent which emerged from these studies is dibenzyl phosphorochloridate $(C_6H_5CH_2O)_2POCl$. This rather unstable substance is readily prepared in solution from dibenzyl phosphite by chlorination either with chlorine itself or with *N*-chlorosuccinimide, and it reacts smoothly with alcohols in presence of tertiary bases to yield alkyl dibenzyl phosphates from which the protecting groups may be removed by a variety of methods. Monodebenzylation may be effected by treatment with strong tertiary bases, by anionic fission, or by partial hydrogenation; complete debenzylation can be brought about by e.g. hydrolysis, catalytic hydrogenolysis, or ammonolysis. Although dibenzyl phosphorochloridate has

been the most generally used reagent for the phosphorylation of alcohols, other methods have been developed by us which are also effective and find uses in particular cases. These include phosphorylation with tetra-esters of pyrophosphoric acid, with mixed anhydrides of diesters of phosphoric acid with stronger acids (e.g. sulphonic acids), the reaction of dialkyl phosphites with polyhalogen compounds in presence of a base and the substance to be phosphorylated, and the important phosphite route in which an alcohol is converted first to an alkyl benzyl phosphite by treatment with the mixed anhydride of benzyl phosphorous acid and diphenyl phosphoric acid, the phosphite then being converted to phosphate either *via* the phosphorochloridate or by direct oxidation. As a result of these basic studies on phosphorylation we were able not only to synthesize all the theoretically possible simple nucleotides derived from the natural ribonucleosides and deoxyribonucleosides but to open the way to an attack on the more complex problems presented by the nucleic acids and the nucleotide coenzymes. Since these represent two distinct though interrelated aspects of nucleotide chemistry it will be convenient to treat them separately in this discussion.

Nucleic acids

It is now well over eighty years since the first nucleic acid was isolated by Miescher from pus cells. Since that time it has become clear that they are essential constituents of all living cells, commonly occurring in association with proteins in the so-called nucleoproteins among which are numbered many viruses and enzymes, and that they are among the most complex of all the substances occurring in living matter. Over the years many early misconceptions have been removed and it is now recognized that the number of individual nucleic acids is large and that all so far discovered belong to one or other of two types – the ribonucleic acids and the deoxyribonucleic acids, the former yielding on hydrolysis D-ribose and the latter 2-deoxy-D-ribose.

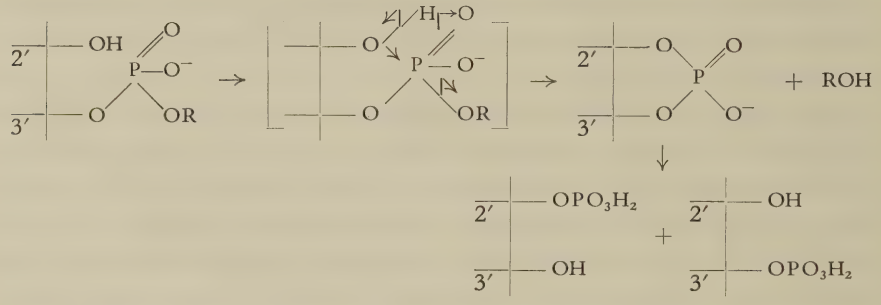
The general nature of the nucleic acids is indicated by their behaviour on hydrolytic breakdown, when they yield simple nucleotides which can be hydrolysed further to give nucleosides and phosphoric acid in equimolecular proportions. The nucleic acids are thus to be regarded as polynucleotides in which, as Levene and Simms showed, individual nucleotides are linked one to the other through phosphodiester groups – pyrophosphate and ether link-

ages are absent. Although the nucleic acid molecules are very large – molecular weights may be as high as several million in some cases – the number of individual nucleosides involved in them is surprisingly small. Indeed from the ribonucleic acids, in general 4 nucleosides are obtained – adenosine, guanosine, uridine, and cytidine – although recently there have been reports of trace amounts of one or two other nucleosides in certain individual acids. Deoxyribonucleic acids yield 4 main nucleosides – deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine – but at least two others, 5-methyl- and 5-hydroxymethyl-cytidine, are also of fairly frequent occurrence. Knowing from our initial studies the complete structure of the nucleosides the outstanding problem in nucleic acid chemistry was the precise location of the internucleotidic linkage.

Until 1949 it was believed that alkaline hydrolysis of ribonucleic acids yielded only four simple nucleotides, which, mainly on evidence adduced by Levene, some of which we now know to have been of doubtful validity, were regarded as the 3′-phosphates of the respective nucleosides. On this basis it was not at all easy to provide a rational polynucleotide structure. In 1949, however, Cohn began to apply the technique of ion-exchange chromatography to alkaline hydrolysates of ribonucleic acids, and he showed that they contained not four, but eight, nucleotides made up of four pairs of isomeric nucleotides, each pair derived from one of the four nucleosides; these pairs he described originally as the *a* and *b* nucleotides, i.e. there were adenylic acid *a* and adenylic acid *b*, uridylic acid *a* and uridylic acid *b*, etc. By a fortunate coincidence this work was carried out at much the same time as we were carrying out the synthesis of the individual ribonucleotides and it soon became clear that the *a* and *b* nucleotides were, in fact, the 2′- and 3′-phosphates of the respective nucleosides although at the time we were unable to say with any certainty which was the 2′- and which the 3′-derivative. It will simplify our story if I say here that we were able later to establish firmly that Cohn's *a* nucleotides are the 2′-phosphates and his *b* nucleotides the 3′-phosphates of the respective nucleosides, but the reasons for our inability to differentiate them immediately are at once interesting and of profound significance for the understanding of nucleic acid structure and behaviour, and have had wide repercussions in other areas of phosphate chemistry.

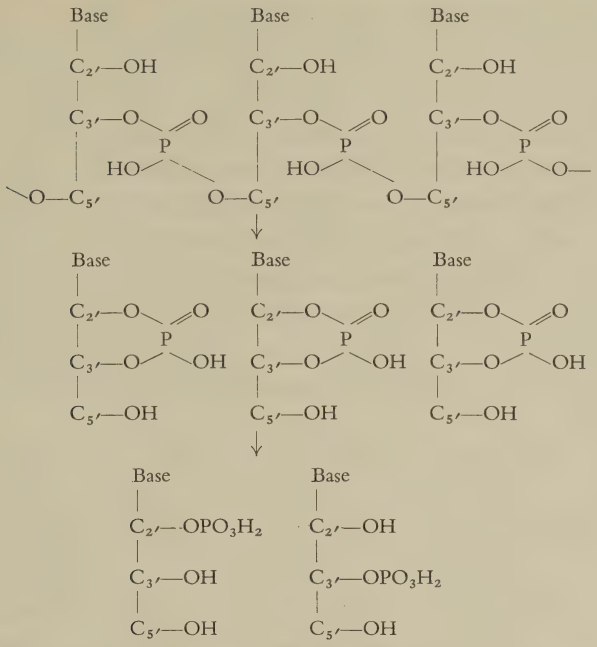
Our early attempts to prepare 2′- or 3′-nucleotides individually by phosphorylation of ribonucleoside derivatives failed partly because of difficulties in group protection but more particularly because of phosphoryl migration which always led to mixtures of the 2′- and 3′-isomers being obtained. Al-

though stable in alkaline solution, either pure isomer is converted in acid solution by phosphoryl migration into an equilibrium mixture of the two, probably by way of a cyclic intermediate. Even more interesting is the behaviour of mono-esters of the 2′- and 3′-nucleotides. Normally, phosphodiesters are remarkably stable towards alkali, but these esters are not. They hydrolyse readily in dilute aqueous alkali yielding always a mixture of both isomeric nucleotides. The mechanism of this hydrolysis is, we believe, that pictured below (only the 2′- and 3′-positions of the sugar residue are here shown since the rest of the molecule is irrelevant to our argument).



It should be noted that the overall reaction as indicated above is a two-stage process; the first is formation of the cyclic phosphate with simultaneous expulsion of the group R, and the second the hydrolysis of the cyclic phosphate in either of two ways to give a mixture of nucleotides. It may also be mentioned that the cyclic phosphates have indeed been isolated as first products in the above hydrolysis and have also been prepared synthetically and shown to have the expected properties. This interesting behaviour is, of course, not confined to the esters of the 2′- and 3′-nucleotides; it is indeed observed in all phosphodiesters where a hydroxyl occurs *cis* to the phosphate group on the vicinal carbon atom. An analogous behaviour is shown, for example, by the mono-esters of the glycerophosphoric acids. But it is of particular relevance to an understanding of nucleic acid behaviour, for, if in the abbreviated formula of the nucleotide ester used above, the group R represents a polynucleotide chain, then the analogy with ribonucleic acid itself becomes clear. The following scheme (in which the expression Base—$C_{2'}$—$C_{3'}$—$C_{5'}$ is used to represent a ribonucleoside residue, 2′, 3′ and 5′ being the only possible points of attachment of phosphate) represents our view of the hydrolytic breakdown of a ribonucleic acid with alkali to give exclusively a mixture of the 2′- and 3′-phosphates of the ribonucleosides.

This theory of hydrolytic breakdown provides a simple explanation for the



fact that ribonucleic acids yield simple nucleotides with alkali, no larger fragments being obtained, and also explains why, in contradistinction to ribonucleic acids, the deoxyribonucleic acids are alkali stable; lacking the vicinal hydroxyl group on $C_{(2')}$, the latter compounds show the normal stability of simple di-esters of phosphoric acid. On these considerations, coupled with certain other evidence, Brown and I postulated both types of nucleic acids as 3′:5′-linked polynucleotides. All subsequent work has confirmed this view of their structure and it is to-day generally accepted. The main evidence as now available may be briefly summarized for the two types of nucleic acid as follows:

Ribonucleic acids. Unstable to alkali yielding simple nucleotides; the cyclic phosphates postulated as intermediates have been in fact isolated. Enzymic hydrolysis (snake venom) shows the participation of $C_{(5')}$ in the internucleotidic linkage. Studies of the action of ribonuclease and spleen nuclease on the cyclic 2′:3′-phosphates of nucleosides and on esters of 2′- and 3′-nucleotides shows that only $C_{(3')}$ is involved in the internucleotidic linkage. This is supported also by the behaviour of synthetic dinucleoside phosphates (5′:5′, 2′:5′ and 3′:5′) towards chemical and enzymic hydrolysis.

Deoxyribonucleic acids. Stable to alkali; hydrolysis by appropriate enzymes can yield 3′- or 5′-nucleotides. Acid hydrolysis yields *inter alia* pyrimidine

nucleoside 3′: 5′-diphosphates. Moreover dithymidine-3′:5′-dinucleotide occurs in digests of deoxyribonucleic acids and has been identified with a synthetic specimen.

It seems probable that the nucleic acids are linear rather than branched polyesters. This is certainly true of isolated deoxyribonucleic acids, since only branching on phosphorus is theoretically possible and such branching points involving phosphotriester linkages would not have the necessary alkali-stability. In the case of ribonucleic acids we have shown that branching on phosphorus is incompatible with stability requirements, but chain branching on $C_{(2')}$ of the sugar residue of the nucleoside remains a theoretical possibility although no experimental evidence for its occurrence in any ribonucleic acid has yet been adduced.

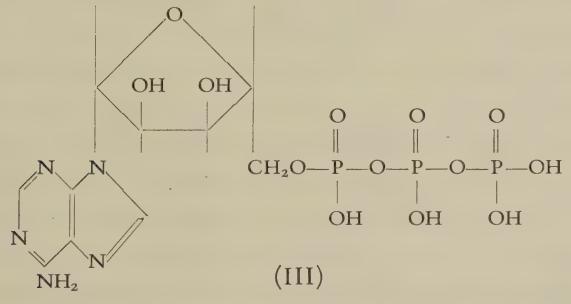
Since the essential difference between individual nucleic acids must reside in the different sequence of nucleoside residues in them, methods for sequence determinations are clearly of importance for further work. Studies on this problem have not been encouraged by the fact that, as yet, no truly homogeneous nucleic acid preparations have been made, but we have devised one method for stepwise degradation which has been shown to be effective for oligonucleotides and may be capable of application to ribonucleic acids themselves.

As yet chemical synthesis in the polynucleotide field is in its infancy. Unambiguous syntheses of dinucleoside phosphates and of at least one dinucleotide have been realized and a start has been made using, essentially, mixed anhydride methods to develop polycondensation methods suitable for rapid polynucleotide synthesis. The increased activity now evident in this field, both in my own laboratories and in those of my former students and colleagues, encourages me to predict rapid advances in this important aspect of nucleic acid research.

Time does not permit me to pursue this topic further and to trace how the chemical information discussed above has been combined with the results of X-ray and other studies to build up current views on the macromolecular structure of the deoxyribonucleic acids. Suffice to say that the double helical structure of the DNA molecule adumbrated first by Watson and Crick on these foundations bids fair to open a new era in molecular biology. For it offers clues to the significance of nucleic acids in the transmission of hereditary characteristics and, taken in conjunction with our greater understanding of the properties and reactions of organic phosphates, it permits an approach to a closer understanding of the role of nucleic acid in cellular processes.

Nucleotide coenzymes

The term nucleotide coenzyme is applied to a large and growing group of substances which are vital components of many enzyme systems involved in metabolic processes. They function in association with specific proteins or apoenzymes, the complete enzyme system being made up of the combination apoenzyme + coenzyme (v. Euler). Historically, the first member of the group is cozymase or diphosphopyridine nucleotide (DPN) whose existence was recognized in 1906 by Harden and Young, although it was not in fact isolated in a pure state until 1936 (v. Euler and Schlenk); it functions as coenzyme in a group of oxidation-reduction enzymes belonging to the pyridinoprotein group. Other examples are flavin-adenine-dinucleotide (FAD) found in many flavoproteins, adenosine triphosphate (ATP) acting as a cophosphorylase and also as a provider of the energy used in muscular contraction and many others. All known members of the group belong to one or other of two types: (a) monoesters of polyphosphoric acids in which the esterifying group is a nucleoside derivative, or (b) unsymmetrical P^1P^2-diesters of pyrophosphoric acid in which at least one of the esterifying groups is a nucleoside derivative. Adenosine triphosphate (III) is an example of type (a) and cozymase or DPN (IV) of type (b).



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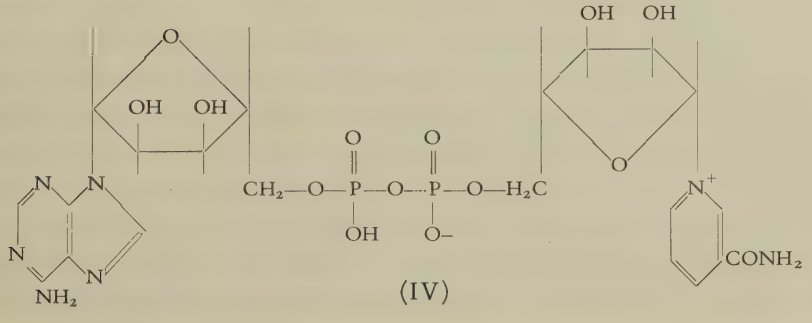
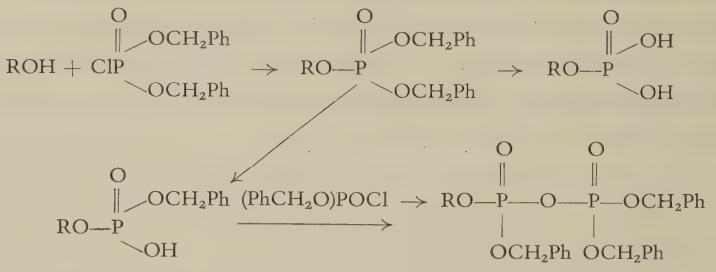


(IV)

An examination of structures (III) and (IV) at once reveals the three basic problems of nucleotide coenzyme synthesis: (1) the synthesis of nucleosides, (2) the phosphorylation and polyphosphorylation of nucleosides, and (3) the linkage of dissimilar molecules one to another by pyrophosphate residues. Of these problems, solutions to (1) and to the simple phosphorylation part of (2) were available to us from the work I have already described. Here I shall discuss only the synthesis of polyphosphates and of unsymmetrical diesters of pyrophosphoric acid leading to actual coenzyme synthesis.

Starting from our most frequently used phosphorylating agent, the simplest and most direct route to pyrophosphates is the reaction between dibenzyl phosphorochloridate and the salt of a phosphodiester. With the variety of procedures open to us for partial or complete removal of benzyl groups, the scheme shown schematically below can give not only monoesters of phosphoric and pyrophosphoric acids but also, by simple extension, either mono- or di-esters of polyphosphoric acids in general.



This simple method of polyphosphate synthesis was used by us in the first total syntheses of adenosine-5′-pyrophosphate (ADP) and adenosine-5′-triphosphate (ATP). The yields obtained in these early syntheses were usually poor for reasons which shortly became apparent to us as efforts to discover other and better procedures increased our knowledge of the properties and reactions of esters of pyrophosphoric acid.

Fully esterified pyrophosphates are very labile substances. Not only do they phosphorylate amines and alcohols but they readily undergo exchange reactions with other anions. For example, in presence of a base, tetraphenyl pyrophosphate reacts with dibenzyl phosphoric acid very rapidly even at 0° to yield tetrabenzyl pyrophosphate and diphenyl phosphoric acid; this reaction is clearly a two-stage nucleophilic substitution, the first stage being formation of the unsymmetrical ester P^1-diphenyl P^2-dibenzyl pyrophosphate. The reaction appears to be a general one for pyrophosphates and for

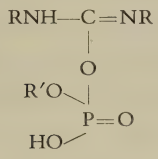
mixed anhydrides of phosphoric with other acids. In its most simplified form we may say that if two acids A and B form an anhydride AB, then if AB is brought into contact with the anion of an acid C then if A is stronger (i.e. has a more stable anion) than B, and C is weaker than A we will have the reaction AB + C→ BC + A. It is this reaction that is primarily responsible for the low yields obtained in the ADP and ATP syntheses just mentioned, for the initial products of reaction are fully esterified polyphosphates and as such they rapidly undergo exchange reactions leading to reduced yields and to complex and difficultly separable mixtures. This tendency to undergo exchange reactions is less evident in the partially esterified polyphosphates (which are correspondingly less effective phosphorylating agents) and the phosphorochloridate method of polyphosphate synthesis can be considerably improved if partially esterified phosphates are employed as starting materials. By this type of procedure we were able to effect total syntheses of the coenzymes flavin-adenine-dinucleotide (FAD), uridine-diphosphate-glucose (UDPG) and uridine-diphosphate-galactose (UDPGal), as well as to provide much improved syntheses of the nucleoside-5′ polyphosphates.

The exchange reaction of polyphosphates which I have mentioned above is of particular interest because I have no doubt that it represents the main reaction employed in nature for polyphosphate synthesis. Numerous examples could be given but one will suffice. The enzymic synthesis of FAD from riboflavin phosphate and ATP described by Kornberg is clearly a reaction of this type. It is interesting to note that in such cases Nature appears to use mono-esterified pyrophosphates whereas in the laboratory analogous reactions seem to occur readily only with fully esterified compounds. Presumably the protein component of the enzyme involved in the biological reaction produces an effect on the polyphosphates akin to that brought about by esterification. This labilizing effect of the enzyme protein may well be analogous to the labilization of the terminal phosphate residue of ATP which we have found can be achieved by allowing it to form an inclusion compound with β-cyclodextrin, and if so it suggests a new approach to some of the problems of enzyme action and specificity. In the laboratory the exchange reaction, using fully esterified pyrophosphates, has not proved very useful for coenzyme synthesis since the extreme lability of the initially formed products makes the reaction too difficult to control.

Quite apart from difficulties caused by the occurrence of exchange reactions, the phosphorochloridate route has also the disadvantage that, where nucleoside phosphorochloridates are employed, protection of the sugar hy-

droxyl groups by acylation or alkylation is necessary and, moreover, non-hydroxylic solvents must be used since phosphorochloridates react very readily with alcohols and with water. We have therefore devoted a good deal of effort to developing reagents for synthesizing pyrophosphates from phosphates without the need for protecting groups and which might be used even in presence of water since nucleotides are most easily handled in polar and particularly aqueous solvents. This has meant, in fact, the use of reagents which react with acids to form anhydrides more readily than they will react with water. The first successful reagents of this type which we used were the dialkyl and diaryl carbodi-imides (RN=C=NR). These substances – the one most frequently employed has been dicyclohexyl carbodi-imide – react smoothly with mono- and di-esters of phosphoric acid to yield respectively di- and tetra-esters of pyrophosphoric acid and they do so even in the presence of moderate amounts of water provided that excess of the carbodi-imide is employed. The reaction goes well both in polar and non-polar media and, although the mechanism has not been studied in detail, the first step is almost certainly an addition of phosphate to the carbodi-imide to give an adduct of type (V), which is then attacked by phosphate anion to give a pyrophosphate and the dialkyl urea. The overall reaction is very rapid and it has not been possible to halt it at the ψ-urea phosphate (V) stage; as a result, although the reaction is well-nigh ideal for the production of symmetrical pyrophosphates, it is less satisfactory for unsymmetrical pyrophosphates of the nucleotide coenzyme type, since treatment of a mixture of two different phosphates with a carbodi-imide normally gives a mixture of all possible symmetrical and unsymmetrical pyrophosphates, the resolution of which into its components is often a matter of considerable difficulty. Despite this defect we have used the carbodi-imide reaction to synthesize a variety of nucleoside polyphosphates, uridine-diphosphate-glucose (UDP) and most recently to effect the total synthesis of cozymase (DPN) and of triphosphopyridine nucleotide (TPN). It is of some interest to note that in the cozymase synthesis from nicotinamide nucleotide and adenosine-5′ phosphate using di*cyclo*hexyl carbodi-imide very little di-(nicotinamide nucleoside-5′) pyrophosphate was produced with a corresponding increase in the yield of the desired unsymmetrical product (DPN). Although the reason for this unexpectedly favourable result is not yet fully understood, it may be a characteristic of carbodi-imide reactions involving dipolar-ionic molecules, since a similar result has been obtained by Kennedy in his preparation of cytidine-diphosphatecholine.

In addition to the carbodi-imides, several other reagents have been studied, which, although in general somewhat less reactive, function in the same way and are of practical value in certain cases. These reagents include the ketenimines, cyanamide and the dialkyl cyanamides. All these share with the carbodi-imides the disadvantage of producing mixtures of products when applied to unsymmetrical pyrophosphate synthesis; the dialkylcyanamides, however, are of particular value when it is desired to operate in aqueous solvents since they are considerably more resistant to hydrolysis than the carbodi-imides.



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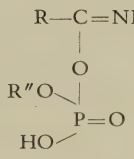

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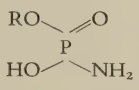


(VII)

We have devoted much effort to devising other reagents and methods for pyrophosphate synthesis specifically designed to produce unsymmetrical esters and so to avoid the waste of materials and the inconvenience of the carbodi-imide method. Of these I shall mention only briefly two interesting methods which have emerged – the imidoyl phosphate method and the phosphoramidic acid method. The first of these employs as reagents imidoyl phosphates (e.g. VI) for which several preparative methods have been devised. These substances, which bear some formal resemblance to the enolphosphates occurring in nature, react readily enough with phosphate anions to give pyrophosphates; unfortunately, however, in polar media some exchange between phosphate and imidoyl phosphate occurs and mixed products are again obtained. Similarly mono-esters of phosphoramidic acid (e.g. VII), when protonated, react readily with phosphoric acids to yield pyrophosphates; they can be used to excellent effect in the synthesis of monoesters of polyphosphoric acids (e.g. of ADP and ATP) but once again they yield mixtures of symmetrical and unsymmetrical esters when applied to the synthesis of diesters of pyrophosphoric acid.

In this field of nucleotide coenzyme synthesis, we are thus still seeking an ideal method for unsymmetrical pyrophosphate synthesis, although having already developed five distinct types of synthetic method we have reached a point at which the synthesis of any coenzyme molecule can be undertaken with reasonable certainty of success. Our practical interest in the field to-day

lies very much in the methods of pyrophosphate formation and in the behaviour of our pyrophosphates and mixed anhydride intermediates. For in the properties of such anhydrides lies the secret of many biological processes and it is noteworthy that in our search for new methods we are in many ways coming closer and closer to the methods of Nature – not only by working in aqueous media but also by using in some of our synthetic routes intermediates which bear a striking resemblance to some of the reactive phosphate derivatives such as enol-phosphates which are widespread in living organisms.

To-day the nucleotides occupy a prominent place in chemical, biochemical and biological research and new vistas are opening before us which may in a relatively short time lead to a far deeper understanding of the mechanisms of the living cell than seemed possible only a few years ago. And this is surely a matter of profound importance to humanity in its ceaseless struggle against disease. We still have far to go, but if, in a small way, our chemical researches contribute to such an understanding, then my colleagues and I are more than satisfied. I specially include my colleagues in this statement, for the work I have attempted to review would not have been possible without the untiring efforts of a large number of brilliant and enthusiastic students and co-workers, among whom I would mention particularly Lythgoe, Baddiley, Atherton, Kenner, Michelson, Brown, Clark, and Webb. It has been a privilege to be associated with them, and to all of them I owe a deep debt of gratitude.

Biography

Sir Alexander Robertus Todd was born in Glasgow on October 2, 1907, the elder son of Alexander Todd, a business man of that city, and his wife Jean Lowrie. He was educated at Allan Glen's School and Glasgow University, where he took his B.Sc. degree in 1928 and, after a short initial research training with T. S. Patterson he proceeded to the University of Frankfurt-on-Maine. Here he studied under W. Borsche and obtained his Ph.D. (Dr. Phil. nat.) in 1931 for a thesis on the chemistry of the bile acids.

Returning to England he worked from 1931–1934 on anthocyanins and other colouring matters with Sir Robert Robinson, the Nobel Prize winner, and took a Ph.D. degree at Oxford University in 1933.

Todd went back to Scotland in 1934 when he joined the staff of Edinburgh University under G. Barger. Two years later, i.e. in 1936 he moved to the Lister Institute of Preventive Medicine, Chelsea, and became Reader in Biochemistry in the University of London in 1937.

In 1938 he was appointed as Sir Samuel Hall Professor of Chemistry and Director of the Chemical Laboratories of the University of Manchester, which position he held until 1944, when he accepted an appointment as Professor of Organic Chemistry at Cambridge University and Fellow of Christ's College.

Todd's work has gained him recognition in many universities and countries. He holds the D.Sc. degree of Glasgow University and has had bestowed upon him honorary doctorates from the Universities of Kiel (Dr. rer. nat.), Glasgow (LL.D.), Hon. D.Sc. London (1958), Madrid (1959), Exeter (1960) Leicester (1960), Aligarh (1960), and in 1961 Wales, Yale and Sheffield; also Hon. LL.D. from Melbourne in 1960. He is a Fellow of the Royal Society, foreign member of the National Academy of Sciences, the American Academy of Arts and Sciences, the Austrian Academy of Sciences and the Spanish Council of Scientific Investigation, and an honorary member of the French German and Spanish chemical societies and member of the Deutsche Akad. Naturforscher Leopoldina, Halle, (1959). He holds the Meldola Medal of the Royal Institute of Chemistry and the Society of Maccabeans; the Davy Med-

al and Royal Medal of the Royal Society, the Cannizaro Medal of the Italian Chemical Society and the Lavoisier Medal of the French Chemical Society. He has been Tilden Lecturer and Pedler Lecturer of the Chemical Society, Bakerian Lecturer of the Society of Chemical Industry, also visiting professor at California Institute of Technology (1938), the University of Chicago (1948), Sidney University (1950), Massachusetts Institute of Technology (1954) and the University of California (1957). He was elected Hon. Member, New York Academy of Sciences (1959), Hon. Fellow of the Royal Australian Chemical Institute (1960), President of the Chemical Society, London, 1960–1962, Master of the Worshipful Company of Salters, 1961–1962.

Todd has taken considerable interest in international scientific affairs; he is President of the International Union of Pure and Applied Chemistry, and Chairman of the British National Committee for Chemistry. He has served on many Government Committees and in 1952 was elected Chairman of the British Government's Advisory Council on Scientific Policy. He is a Managing Trustee of the Nuffield Foundation.

The main subjects of Todd's researches have been the chemistry of natural products of biological importance and, in addition to the nucleotide and nucleotide coenzyme studies described in his Nobel Lecture, the chemistry of vitamins B_1, E and B_{12}, the constituents of *Cannabis* species, insect colouring matters, factors influencing obligate parasitism and various mould products.

Knighted in 1954, he was raised to the Peerage in March, 1962, being created Baron Todd of Trumpington.

Lord Todd is married to Alison Sarah, daughter of Nobel Prize winner Sir Henry Dale, and they have a son, Alexander Henry, and two daughters, Helen Jean and Hilary Alison.

Chemistry 1958

FREDERICK SANGER

«for his work on the structure of proteins, especially that of insulin»

Chemistry 1958

Presentation Speech by Professor A. Tiselius, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Your Royal Highnesses, Ladies and Gentlemen.

The proteins are among the most complicated and enigmatic substances in Nature and appear to be particularly closely related to all that we call Life. To this group of key substances belong for example all enzymes and many hormones, which control the chemical processes of Life, also the viruses and many toxins which cause disease, and antibodies, which are formed on vaccination, and are able to protect us against infection. In blood and in all tissues of the body, in muscles, nerves and skin, proteins form an essential and functional constituent. It is the chemical individuality of proteins which is responsible for the species' differences among all living things. The determination of the exact building-plan for these complicated giant molecules appears as one of the greatest problems in today's scientific research.

Even if some protein molecules are big enough to be observed in our most powerful electron microscopes it is not possible by any direct method to see the finer details in their structure. Here one must resort to the indirect methods which chemists use in studying the structure of complicated substances. Thus one has to break down the big molecules by suitable methods and look for simpler and well-known substances among the fragments obtained. This procedure was used with proteins by the German chemist and Nobel laureate Emil Fischer in the beginning of this century. He found that protein molecules contain long chains of so-called amino acids. These are comparatively simple substances of which about 25 different kinds are found in Nature, and they are formed when proteins are boiled with strong acids. Thus we know that proteins contain a large number of different kinds of amino acids, but the composition and, above all, *the sequence* of the amino acids in the chains vary considerably. As a matter of fact it has long been assumed that it is this sequence which determines the individual chemical and physiological properties of different proteins.

That insulin is a physiologically important hormone which is used in the treatment of diabetes is well known to all. Insulin is also a protein and even if its molecules do not belong to the largest, they are sufficiently complicated

to make the task of determining their structure appear a formidable one. It was this venture, however, which Frederick Sanger started fifteen years ago and which after zealous and persistant work gradually led him to a successful solution of the problem; namely the exact mode in which the 51 amino acids of the insulin molecule are linked together.

The start was promising. Sanger developed a method to « mark » at its free amino end the particular amino acid which sits at the end of a chain. For this purpose he used a dye reagent, dinitrofluorbenzene, which is bound comparatively firmly to the amino acid and will remain so even if the chain is broken and the terminal amino acid thus set free. In the complicated mixture of amino acids which results if insulin, « marked » in this fashion, is boiled with acids, one can thus isolate the coloured components, which must represent terminal groups. In this way Sanger could demonstrate that the insulin molecule contains two different chains with different end-groups, and he managed to isolate them after breaking the molecule by oxidation. Thus the problem was simplified: instead of one molecule with 51 amino acids Sanger now had two with 31 resp. 20.

If the chains are only partially broken down – for example by treatment with weak acids or enzymes – one obtains larger fragments of the chain, containing, 2, 3, 4, 5 or more amino acids in the very sequence in which they occur in the intact molecule. Sanger succeeded in isolating and identifying a large number of such fragments from the complicated mixtures obtained by such a treatment. In this work he combined in a very skilful manner different chromatographic and electrophoretic methods, especially paper chromatography as introduced by Martin and Synge, Nobel laureates in Chemistry 1952. Sanger managed to determine the sequence of amino acids in each bit of the chain thus isolated. In this work his « end-group » methods, already mentioned here, was of great help. Each piece represents a number of links in the chain and it now remained to fit all these pieces together in a correct way and thus to reconstruct – on paper at least – the original chain. This part of the work reminds one of laying a puzzle. It was a difficult and painstaking operation but it worked: it proved possible to make the puzzle fit. In other words, Sanger succeeded in reconstructing first one chain and then the other from all the pieces obtained, and particularly important – the result was the same, independent of the method used for breaking the chains.

Thus, Sanger could give the exact sequence of the 31 amino acids in one chain and the 20 in the other. Already earlier he had shown that the two chains are held together to form an insulin molecule with the aid of two

bridges of sulphur atoms. The exact positions of these bridges were determined in a way similar to that used for determination of the structure of the chains.

The structure of insulin had thus been established – a remarkable achievement, indeed. Insulin is a protein, and this was the first time one had succeeded in determining the structure of a substance belonging to this very important group.

The significance of Sanger's work reaches, however, much further. The procedure he has used so successfully can be applied to proteins in general, in attempts to determine their structure. Already now, many research workers are engaged in such investigations, and important results appear to be on the way in the exploration of how proteins play their role as key substances in the chemical processes of Life.

Doctor Frederick Sanger. It sometimes happens that an important scientific discovery is made so to say « overnight » – if the time is ripe and the necessary background is there. Yours is not of that kind. The first successful determination of the structure of a protein is the result of many years of persistant and zealous work, in which the final solution of the problem has been approached step by step. You knew when you began to look into the structure of the insulin molecule 15 years ago that the problem was a formidable one. So did the whole scientific world. Those who knew you, were confident, however, that you would ultimately succeed, and each successive publication from your laboratory strengthened our confidence. Intelligence, knowledge and skill in the mastering of the methods required – we know you have them all – but in such a venture these are not enough. Without your wholehearted devotion to the task you had set before you, many obstacles on your way would have appeared insurmountable. Now that many years of work have been crowned with success you may look back and rejoice. You can also enjoy the satisfaction of seing the roads you have broken and paved being used by many in their search for the building principles of the key substances of Life. However, very likely you are more apt to look ahead. It was Alfred Nobel's intention that his prizes should not only be considered as awards for achievements done but that they should also serve as encouragement for future work. We are confident that you are a worthy recipient of the Nobel award also in this sense. May we offer you our congratulations.

Frederick Sanger. I now request you to receive from the hands of His Majesty the King the Nobel Prize for Chemistry for the year 1958.

Frederick Sanger

The chemistry of insulin

Nobel Lecture, December 11, 1958

It is great pleasure and privilege for me to give an account of my work on protein structure and I am deeply sensitive of the great honour that has been done to me in recognizing my work in this way. Since the work on insulin has extended over about 12 years it will be necessary to give a somewhat simplified account and to omit most of the work that did not contribute directly to the main problem, the determination of the structure of a protein.

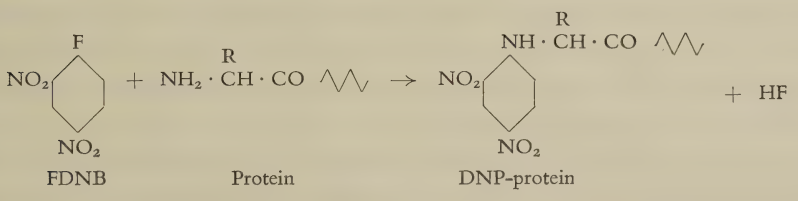
In 1943 the basic principles of protein chemistry were firmly established. It was known that all proteins were built up from amino acid residues bound together by peptide bonds to form long polypeptide chains. Twenty different amino acids are found in most mammalian proteins and by analytical procedures it was possible to say with reasonable accuracy how many residues of each one was present in a given protein. Practically nothing, however, was known about the relative order in which these residues were arranged in the molecules. This order seemed to be of particular importance, since although all proteins contained approximately the same amino acids they differed markedly in both physical and biological properties. It was thus concluded that these differences were dependent on the different arrangement of the amino-acid residues in the molecules. Although very little was known about amino-acid sequence, there was much speculation in this field. The most widely discussed theory was that of Bergmann and Niemann who suggested that the amino acids were arranged in a periodic fashion, the residues of one type of amino acid occurring at regular intervals along the chain. On the other extreme there were those who suggested that a pure protein was not a chemical individual in the classical sense but consisted of a random mixture of similar individuals.

Due largely to the work of Chibnall and his colleagues[1] insulin had been studied in considerable detail. It had a somewhat simpler composition than most proteins, in that two of the commonly occurring amino acids, tryptophan and methionine were absent and an accurate analysis was available.

Moreover, using the Van Slyke procedure, Chibnall had shown that insulin was peculiar in having a high content of free α-amino groups. This indicated that it was composed of relatively short polypeptide chains since free α-amino groups would be found only on those residues (the N-terminal residues) which were present at one end of a chain. Thus the number of chains could be determined from the number of these N-terminal residues. The nature of one of these N-terminal residues was in fact known. Jensen & Evans[2] had shown that the phenylhydantoin of phenylalanine could be isolated from an acid hydrolysate of insulin that had been treated with phenylisocyanate, thus indicating that phenylalanine was at the end of one of the chains. At that time this was the only case where the position of an amino acid in a protein was known.

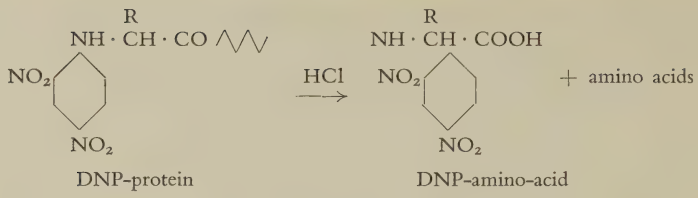
There was considerable doubt about the actual molecular weight of insulin and hence the number of amino acid residues present. Values varying from 36,000 to 48,000 were reported by physical methods but it was shown by Gutfreund[3] that these high values were due to aggregation and it was suggested that the real molecular weight or subunit was 12,000. This indicated that there were about 100 residues in the molecule. More recently Harfenist & Craig[4] have shown that the actual value is about 6,000; however during most of our work it was believed to be 12,000.

In order to study in more detail the free amino groups of insulin and other proteins, a general method for labelling them was worked out[5]. This was the dinitrophenyl (or DNP) method. The reagent used was 1:2:4 fluorodinitrobenzene (FDNB) which reacts with the free amino groups of a protein or peptide to form a DNP derivative:



The reaction takes place under mildly alkaline conditions which normally do not cause any breakage of the peptide bonds.

The DNP-protein is then subjected to hydrolysis with acid which splits the peptide bonds in the chain, leaving the N-terminal residue in the form of its DNP-derivative.



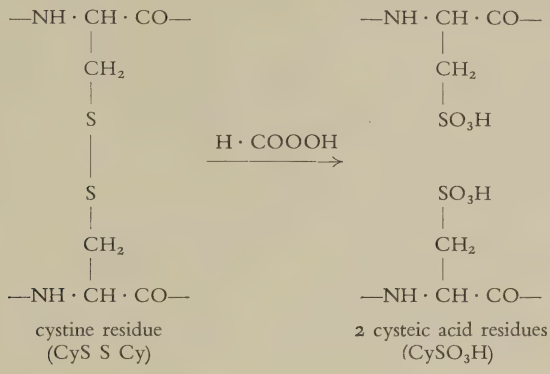
DNP-protein DNP-amino-acid

The DNP-amino acids are bright yellow substances and can be separated from the unsubstituted amino acids by extraction with ether. They could be fractionated by partition chromatography, a method which had just been introduced by Gordon, Martin & Synge[6] at that time. The DNP-amino acids could then be identified by comparison of their chromatographic rates with those of synthetic DNP-derivatives. In the original work on insulin, silica-gel chromatography was used, though more recently other systems, particularly paper chromatography, have been found more satisfactory. Having separated and identified the DNP-derivatives they could be estimated colorimetrically.

When the method was applied to insulin, three yellow DNP-derivatives were found in the hydrolysate of the DNP-insulin. One of these was not extracted into ether and was ε-DNP-lysine which was formed by reaction of the FDNB with the free ε-amino group of lysine residues which are bound normally within the polypeptide chain. The others were identified as DNP-phenylalanine and DNP-glycine, and estimation showed that there were two residues of each assuming a molecular weight of 12,000. This suggested to us that insulin was composed of four polypeptide chains, two with phenylalanine and two with glycine end-groups. This method has now been applied widely to many proteins and peptides, and together with the Edman phenylisothiocyanate method is the standard method for studying N-terminal residues. In general it has been found that the chains of other proteins are much longer than those of insulin. All pure proteins appear to have only one or two N-terminal residues.

It seemed probable that the chains of insulin were joined together by the disulphide bridges of cystine residues. Insulin is relatively rich in cystine and this was the only type of cross-linkage that was definitely known to occur in proteins. It was thus next attempted to separate the peptide chains by splitting the disulphide bridges. Earlier attempts to do this by reduction to —SH derivatives had not proved successful and had given rise to insoluble products which were probably due to some type of polymerization. More satisfactory results were obtained by oxidation with performic acid[7]. The

cystine residues were converted to cysteic acid residues thus breaking the cross-links:



cystine residue (CyS S Cy)

2 cysteic acid residues ($CySO_3H$)

Performic acid also reacts with methionine and tryptophan residues, the two amino acids which fortunately were absent from insulin.

From the oxidized insulin two fractions could be separated by precipitation methods. One (fraction A) contained glycine and the other (fraction B) phenylalanine N-terminal residues. Fraction A was acidic and had a simpler composition than insulin, in that the six amino acids: lysine, arginine, histidine, phenylalanine, threonine, and proline, were absent from it. It thus had no basic amino acids, which were found only in fraction B. From a quantitative determination of the end groups it was concluded that fraction A contained about 20 residues per chain, four of these being cysteic acid and fraction B had 30 residues, two of which were cysteic acid. Since the yield of each fraction was greater than 50% in terms of the N-terminal residues present and since they appeared to be homogeneous it seemed likely that there was only one type of glycyl chain and one type of phenylalanyl chain. This was confirmed by a study of the N-terminal sequences[8].

When the DNP derivative of fraction B was subjected to complete acid hydrolysis, DNP-phenylalanine was produced. If however it was subjected to a milder acid treatment so that only a fraction of the peptide bonds were split, DNP-phenylalanyl peptides were produced which contained the amino acid residues near to the N-terminal end and by an analysis of these peptides it was possible to determine the N-terminal sequence to four or five residues along the chain. The results with fraction B are shown in Table 1. It was concluded from these results that all the N-terminal phenylalanine residues of insulin were present in the sequence Phe · Val · Asp · Glu. This suggested that if there were in fact two phenylalanyl chains, then these two

were identical. Similar results were obtained with fraction A, and it was shown that the N-terminal glycine residues were present in the sequence Gly · Ileu · Val · Glu · Glu.

Table 1.

Peptide	*Products of complete hydrolysis of peptide*	*Products of partial hydrolysis*	*Structure*	*Yield from DNP* insulin*
B1	DNP-phenylalanine	—	DNP-Phe	13
B2	DNP-phenylalanine Valine	B1	DNP-Phe · Val	16
B3	DNP-phenylalanine Valine Aspartic acid	B1, B2	DNP-Phe · Val · Asp	13
B4	DNP-phenylalanine Valine Aspartic acid Glutamic acid	B1, B2, B3	DNP-Phe · Val · Asp · Glu	30
Other bands giving B4 on partial hydrolysis				20
			Total	92

* Moles peptide as per cent of total N-terminal phenylalanine residues of insulin.

These results, besides giving information about the position of certain residues in the polypeptide chains, showed for the first time that the molecule was composed of only two types of chains and that if the molecular weight was 12,000 as was then believed, then the molecule was built up of two identical halves. The other alternative, which was later shown to be the case, was that the actual molecular weight was 6,000. In any case the structural problem was somewhat simplified since we were now concerned with determining the sequence in two chains containing 20 and 30 residues respectively.

The main technical problem was the fractionation of the extremely complex mixtures that resulted from partial hydrolysis of a protein. However Consden, Gordon, Martin & Synge[9] had shown that small peptides could be well fractionated by paper chromatography and had determined the sequence in the pentapeptide « gramicidin-S » from the composition of peptides produced on acid hydrolysis.

At this point (1949) I was joined by Dr. Hans Tuppy who came to work in Cambridge for a year. Although we did not seriously envisage the pos-

sibility of being able to determine the whole sequence of one of the chains within a year, it was considered worth while to investigate the small peptides from an acid hydrolysate using essentially the methods that had been applied to « gramicidin-S ». Studies were initiated on both the chains at the same time but it soon became clear that there would be more difficulties with fraction A although it was the shorter chain and the work on fraction B progressed so favourably and Tuppy worked so hard that by the end of the year we were virtually able to deduce the whole of the sequence of its 30 residues[10].

Fraction B was subjected to partial hydrolysis with acid. Since the mixture was too complex for direct analysis by paper chromatography it was necessary to carry out certain preliminary group separations in order to obtain fractions containing 5–20 peptides that could then be separated on paper. This was accomplished by ionophoresis, ion-exchange chromatography, and adsorption on charcoal. These simplified mixtures were then fractionated by two-dimensional paper chromatography. The peptide spots were cut out and the material eluted from the paper, subjected to complete hydrolysis and analysed for its constituent amino acids. Another sample of the peptide was then investigated by the DNP technique to determine the N-terminal residue. Table 2 illustrates the results obtained with a very acidic fraction obtained by ion-exchange chromatography. This contained only peptides of

Table 2. Cysteic acid peptides identified in a partial acid hydrolysate of fraction B.

(The inclusion of residues in brackets indicates that their relative order is not known.)

	$CySO_3H \cdot Gly$	$CySO_3H \cdot Gly$
	$Val \cdot CySO_3H$	
		$Leu \cdot CySO_3H$
	$Val \cdot (CySO_3H, Gly)$	
		$Leu \cdot (CySO_3H, Gly)$
	$Leu \cdot (Val, CySO_3H)$	
	$Leu \cdot (Val, CySO_3H, Gly)$	
Sequences deduced	$Leu \cdot Val \cdot CySO_3H \cdot Gly$	$Leu \cdot CySO_3H \cdot Gly$

cysteic acid. Since there are only two such residues in fraction B all these peptides must fit into two sequences. The way in which the two sequences $Leu \cdot CySO_3H \cdot Gly$ and $Leu \cdot Val \cdot CySO_3H \cdot Gly$ were deduced from the results obtained with the peptides is illustrated in the table.

In this way about 45 peptides were identified in various fractions of the partial acid hydrolysate and the following five sequences were deduced as being present in the phenylalanine chain.

1. Phe · Val · Asp · Glu · His · Leu · $CySO_3H$ · Gly (N-terminal sequence).
2. Gly · Glu · Arg · Gly.
3. Thr · Pro · Lys · Ala.
4. Tyr · Leu · Val · $CySO_3H$ · Gly.
5. Ser · His · Leu · Val · Glu · Ala.

These five sequences contain all but four of the amino acid residues of fraction B. It was not possible to determine from the small peptides derived from acid hydrolysates the position of the remaining four residues or how the above five sequences were joined together. There were two reasons for this. Firstly there was considerable technical difficulty in fractionating the peptides containing two or more of the non-polar residues such as tyrosine or leucine. It happened that these residues were grouped together in the chain (see below) and gave rise to a mixture of peptides that moved fast on paper chromatograms and were not well resolved. The second difficulty was due to the great lability to acid of the bonds involving the amino groups of the serine and threonine residue. It was never possible to find a peptide containing this bond and hence to know what residue preceded the serine and threonine.

It was thus necessary to use another method of hydrolysis that would show a different specificity from concentrated acid. Hydrolysates prepared by the action of dilute acid at high temperatures or of alkali were studied but yielded little further information. Much more successful however was the use of proteolytic enzymes[11]. Initially we had refrained from using them since it was considered that they might bring about re-arrangement of the peptide bonds by transpeptidation or actual reversal of hydrolysis. Subsequent work has however shown that this is not a very serious danger and in fact proteolytic enzymes are the most useful hydrolytic agent for studies of amino acid sequences.

Proteolytic enzymes are much more specific than is acid since only a few of the peptide bonds are susceptible. They give rise to larger peptides which in general are more difficult to fractionate by paper chromatography. However there are relatively few of them so that the mixtures are less complex. In this initial work we used essentially the same methods for studying the

enzymic peptides as we had used for the acid ones, depending largely on paper chromatography for fractionation, although more recently it has been shown that better separations can be obtained by ion-exchange chromatography and by ionophoresis.

As an example we may consider a peptide Bp3 obtained by the action of pepsin. It had the following composition Phe($CySO_3H$, Asp, Glu, Ser, Gly, Val, Leu, His) of which the most important components are aspartic acid and serine since they occur only once in the chain. Aspartic acid is present only in the N-terminal sequence 1 and serine is in sequence 5. This shows that all of sequence 1 and at least the N-terminal part of sequence 5 is present in peptide Bp3. That none of the other sequences are present follows from the fact that Bp3 contains no arginine (sequence 2), threonine, proline, or lysine (sequence 3) or tyrosine (sequence 4). One may thus conclude that the two sequences are joined together. By studying other peptides obtained by the action of pepsin, trypsin and chymotrypsin it was possible to find out how the various sequences were arranged and to deduce the complete sequence of the phenylalanyl chain which is shown below :

Phe · Val · Asp · Glu · His · Leu · $CySO_3H$ · Gly · Ser · His · Leu · Val · Glu · Ala · Leu · Tyr · Leu · Val · $CySO_3H$ · Gly · Glu · Arg · Gly · Phe · Phe · Tyr · Thr · Pro · Lys · Ala.

In this work many more peptides were studied from both acid and enzymic hydrolysates than were actually necessary to deduce the sequence. This was considered essential since the methods used were new and were qualitative rather than quantitative. The fact that all the peptides fitted into the unique sequence given above added further proof to its validity.

Essentially similar methods were used to determine the sequence of fraction A^{12}. Although the shorter of the two chains, the determination of its structure was more difficult. Fraction B contains several residues that occur only once in the molecule and this helps considerably in interpreting the results, whereas fraction A has only a few such residues and these are all near one end. Also fraction A is much less susceptible to enzymic hydrolysis. It is not attacked by trypsin and there is a sequence of thirteen residues which is not split by chymotrypsin or pepsin either. Considerable difficulty was at first experienced with the cysteic acid peptides. Fraction A contains the sequence $CySO_3H$ · $CySO_3H$ and this gave rise to a number of very water-soluble peptides which would not fractionate easily by paper chromatog-

raphy. However it was found that by paper ionophoresis at pH 2.75 they could be well separated since they were the only acidic peptides present. At this pH, — COOH groups are uncharged, —SO_3H groups carry a negative and —NH_2 groups a positive charge. Peptides without cysteic acid were all positively charged, those with one cysteic acid were neutral and could be separated as a group and those with two cysteic acids were negatively charged. If a slightly higher pH (3.5) is used for the ionophoresis, the —COOH groups become slightly charged and all the peptides containing one cysteic acid residue move slowly towards the anode and can be fractionated in this way. This method was found very useful for the separation and identification of cysteic acid peptides. Fig. 1 is a tracing of an ionogram of an acid hydrolysate of fraction A carried out in this way.

The sequence of fraction A was found to be :

Gly · Ileu · Val · Glu · Glu · $CySO_3H$ · $CySO_3H$ · Ala · Ser · Val · $CySO_3H$ · Ser · Leu · Tyr · Glu · Leu · Glu · Asp · Tyr · $CySO_3H$ · Asp.

When a protein is hydrolysed with strong acid, it gives rise not only to amino acids but also to a certain amount of ammonia. This is present in the form of amide groups on some of the aspartic and glutamic acid residues. It was thus necessary to determine the position of these groups[13]. This was done by studying the ionophoretic rates and amide contents of peptides derived from enzymic hydrolysates, since the amide groups are not split off by enzymes, whereas they are by acid. The position of the amide groups are indicated in Fig. 2 by the symbols NH_2.

Having determined the structure of the two chains of insulin the only remaining problem was to find how the disulphide bridges were arranged. About this time it was shown by Harfenist & Craig that the molecular weight of insulin was of the order of 6,000, so that it consisted of two chains containing three disulphide bridges, and not of four chains as we had originally thought. The fact that fraction A contained four cysteic acid residues whereas fraction B had only two indicated that two bridges must connect the two chains together and one must form an intrachain bridge connecting one part of the A chain with another part of the same chain.

In order to determine the distribution of the disulphide bridges, it was necessary to isolate from unoxidized insulin peptides containing intact cystine residues. These could then be oxidized to give cysteic acid peptides

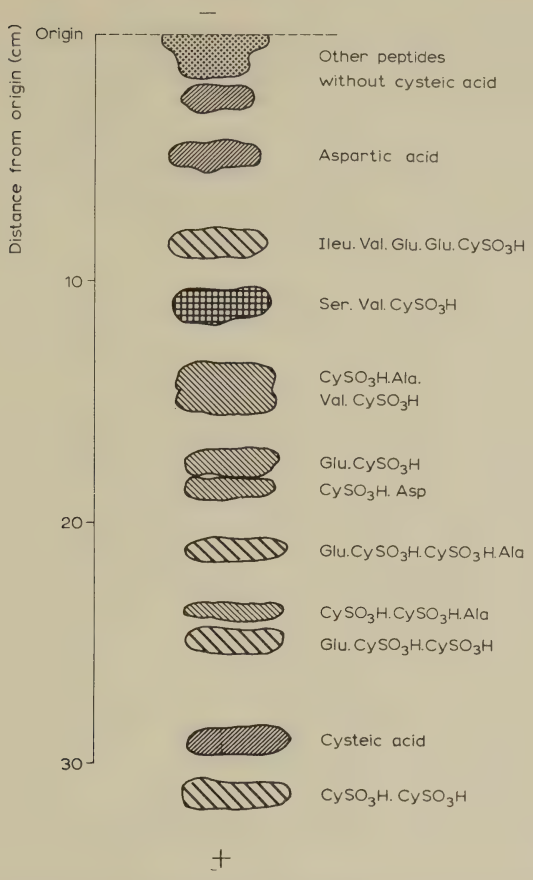
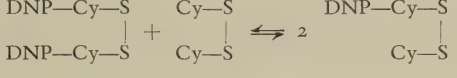


Fig. 1. Ionophoresis of partial acid hydrolysate of fraction A at pH 3.5 showing separation of cysteic acid peptides.

which could be recognized since they had been found in the hydrolysates of the oxidized chains. However an unexpected difficulty arose, in that during hydrolysis a reaction occurred which caused a random rearrangement of the disulphide bonds, so that cystine peptides were isolated which were not actual fragments of the original insulin and it would have appeared from the results that every half-cystine was combined to every other half-cystine residue.

This disulphide interchange reaction could be demonstrated and studied using as a model system a mixture of cystine and bis-DNP cystine, which reacted together to give mono-DNP-cystine



An ether-soluble coloured substance was thus converted to a water-soluble coloured substance and the course of the reaction could be studied by measuring the distribution of colour between ether and water.

It was found that there were two types of disulphide interchange reactions[14]. One took place in neutral and alkaline solution and was catalyzed by —SH compounds. It is probably due to initial hydrolysis of the disulphide which then catalyzes a chain reaction:

$$R_1SSR_2 \xrightarrow{OH^-} R_1S^- + R_2SOH$$
$$R_1S^- + R_3SSR_4 \longrightarrow R_1SSR_3 + R_4S^- \text{ etc.}$$

In neutral conditions the reaction could be inhibited by —SH inhibitors so that it was possible to use enzymic hydrolysis to obtain cystine peptides[15]. Thus for instance with chymotrypsin a peptide was obtained which on oxidation gave the two cysteic acid peptides $CySO_3H \cdot AspNH_2$ and Leu · Val · $CySO_3H$ · Gly · Glu · Arg · Gly · Phe · Phe. The structure of the cystine peptide was thus:

Cy · $AspNH_2$
|
S
|
S
|
Leu · Val · Cy · Gly · Glu · Arg · Gly · Phe · Phe

establishing the presence of a disulphide bridge between the two half-cystine residues nearest the C-terminal ends of the two chains.

It was not, however, possible to determine the positions of the other two disulphide bonds using enzymic hydrolysis, since no enzyme would split between the two consecutive half-cystine residues of the A chain. It was therefore necessary to re-investigate the possibility of using acid hydrolysis.

The disulphide interchange reaction that occurred in acid solution was found to be different from that occurring in neutral and alkaline solution and instead of being catalyzed by —SH compounds, was actually inhibited by them. Not only did this show that a different reaction was involved but it also made it possible to prevent it occurring during acid hydrolysis. Thus when insulin was treated with concentrated acid to which a small amount of thioglycolic acid was added cystine peptides could be isolated which were in fact true breakdown products and from which the distribution of the

remaining two disulphide bonds could be deduced. These are shown in Fig. 2, which shows the complete structure of insulin.

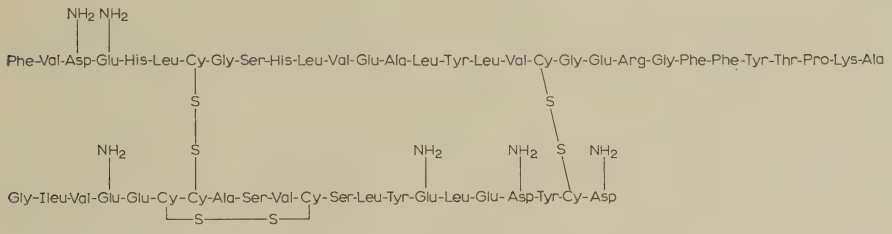


Fig. 2. The structure of insulin.

Of the various theories concerned with protein chemistry our results supported only the classical peptide hypothesis of Hofmeister and Fischer. The fact that all our results could be explained on this theory added further proof, if any were necessary, to its validity. They also showed that proteins are definite chemical substances possessing a unique structure in which each position in the chain is occupied by one and only one amino acid residue.

Examination of the sequences of the two chains reveals no evidence of periodicity of any kind nor does there seem to be any basic principle which determines the arrangement of the residues. They seem to be put together in a random order, but nevertheless a unique and most significant order, since on it must depend the important physiological action of the hormone.

As yet little is known about the relationship of the physiological action of insulin to its chemical structure. One approach to this problem was to study the insulins from different animal species[16,17]. Since all insulins show the same activity it could be concluded that differences would be found only in parts of the molecule that were not important for activity.

All the above results were obtained on cattle insulin. When insulins from four other species were studied by essentially the same methods it was found that the whole of the B chain was identical in all species and the only differences were found in the three amino acids contained within the disulphide ring of the A chain, which in the cattle are Ala·Ser·Val and in the other species are as follows:

Pig	–	Thr · Ser · Ileu
Sheep	–	Ala · Gly · Val
Horse	–	Thr · Gly · Ileu
Whale	–	Thr · Ser · Ileu

These results suggest that the exact structure of the residues in this position is not important for biological activity, but it does not follow that the whole of the rest of the molecule is important.

The determination of the structure of insulin clearly opens up the way to similar studies on other proteins and already such studies are going on in a number of laboratories. These studies are aimed at determining the exact chemical structure of the many proteins that go to make up living matter and hence at understanding how these proteins perform their specific functions on which the processes of Life depend. One may also hope that studies on proteins may reveal changes that take place in disease and that our efforts may be of more practical use to humanity.

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Biography

Frederick Sanger was born on August 13, 1918, at Rendcombe in Gloucestershire, the second son of Frederick Sanger, M.D., a medical practitioner and his wife Cicely. He was educated at Bryanston School and at St. John's College, Cambridge, where he took his B.A. degree in natural sciences in 1939. Since 1940 he has carried out research in the Department of Biochemistry at Cambridge. From 1940 to 1943 he worked with Dr. A. Neuberger on the metabolism of the amino acid lysine and obtained a Ph.D. degree in 1943. From 1944 to 1951 he held a Beit Memorial Fellowship for Medical Research and since 1951 he has been a member of the External Staff of the Medical Research Council. His present position is Head of the Division of Protein Chemistry in the M.R.C. Laboratory for Molecular Biology at Cambridge.

Since 1943 his work has been concerned largely with problems related to the determination of the structure of proteins. These studies resulted in the determination of the structure of insulin.

Sanger was awarded the Corday-Morgan Medal and Prize of the Chemical Society in 1951. In 1954 he became a Fellow of the Royal Society and a Fellow of King's College, Cambridge. He is an Honorary Foreign Member of the American Academy of Arts and Sciences; Honorary Member of the American Society of Biological Chemists, Member of the Academies of Science of Argentina and Brazil, Honorary Member of the Japanese Biochemical Society, and Corresponding Member of the Associatión Química Argentina.

In 1940, he married Margaret Joan Howe; they have two sons and one daughter.

Chemistry 1959

JAROSLAV HEYROVSKÝ

«for his discovery and development of the polarographic methods of analysis»

Chemistry 1959

Presentation Speech by Professor A. Ölander, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Royal Highnesses, Ladies and Gentlemen.

Analytical chemistry is a science, fundamental not only to the other branches of scientific chemical research, but also to applied chemistry, the chemical industry. Further it is important for the other natural sciences, both within inorganic and organic Nature, for medical research, and for many humanistic and even jurisprudential sciences.

The striving of analysts is not only to develop methods yielding accurate results, but even more important for practical work is that the analysis can be carried out rapidly, that it can be done using as small samples as possible, and that very small percentages of various substances can be detected and ascertained.

Polarography is one of these micro-methods which are available to the modern analyst.

Professor Bohumil Kučera of Prague once suggested to the young Jaroslav Heyrovský that he should study certain irregularities in connection with the capillarity of mercury and attempt to disclose their origin. This was one of the innumerable small problems constituting science. Heyrovský let the mercury flow through a glass capillary and weighed the drops. It was a slow and tedious method, and he resolved instead to measure the electric current obtained when he put a tension between the mercury in the capillary and that collecting at the bottom. The glass capillary does not terminate in the air, but in a solution, through which the current now will flow.

Heyrovský found that this device could be used for something much more important than the original problem. It could be used for ascertaining very small quantities of the most diverse substances dissolved in the water, and moreover, to measure their percentages.

Important new discoveries are found where they are not expected. Spectacular advances in our time have been achieved by great teams, and some people are saying that only teamwork is worth-while, whereas a single scientist nowadays can do nothing. Well, teamwork can be efficient, and is organized when you have been able to set an aim for the work. But the new

discoveries are made by some scientist who noticed something strange, possibly by two, independently and in different countries. Then it is important that future team leaders and authorities granting funds do not keep him too strictly to attend his job, but give him a chance to pick up the unexpected new things, in spite of the chance of finding them being diminutive.

When Heyrovský put a small electric tension between the dropping mercury and that collecting at the bottom, he found in accordance with earlier experience that the current increased by steps when the tension was raised over certain fixed values. Earlier people usually introduced the current into the solution by means of a platinum foil. But various substances will stick to the surface of a solid, disturbing the course of the experiment. Because the mercury drop is falling off after a few seconds and a new one then will be forming, this method always ensures us a new clean surface against the solution, and disturbances are avoided.

The current will increase perceptibly even at very small percentages of such substances that will undergo a chemical reaction at the surface of the mercury drop when the current passes. The increase will not be unlimited, but is proportional to the percentage. The tension needed is a characteristic of each substance, and therefore the method gives us information concerning both which substances are present in the solution and their quantities.

Heyrovský together with his Japanese collaborator Shikata built an apparatus which registered how these electric currents varied with the tension applied. This apparatus, named the polarograph, traces a curve, from which can be read both place and height of the various steps.

About a decade elapsed before the method found some use outside Heyrovský's own laboratory. But when this at last happened, people found that for example impurities of a metal sample could be easily and accurately ascertained. Earlier, this problem, of great importance to industry, often was very laborious, time-consuming and the results uncertain.

Heyrovský and his collaborators, at home and abroad, disclosed the theoretical foundations of the methods and worked out its applications to more and more types of problems. Almost all chemical elements can be analysed with the aid of the polarographic method and in Organic Chemistry it is equally useful for the most diverse groups of substances.

Heyrovský also has elaborated modifications of his method which are extremely valuable for special kinds of investigations. For example, it is possible to squirt a jet of mercury into the solution instead of dropping it and the registration will be effected with an oscillograph. But, when speaking

of polarography, one primarily thinks about the classical polarograph and its step curves. A great number of instrument makers the world over are producing these recording instruments, which are nowadays found in every well-equipped analytical laboratory. In contrast to certain other versatile instruments employed in modern analysis, they sell at a reasonable price. Thousands of polarographs scattered over the world facilitate chemical and medical research, and in the industrial laboratories they contribute in cutting prices of both necessities of life and of more advanced results of material production.

Professor Heyrovský. You are the originator of one of the most important methods of contemporary chemical analysis. Your instrument is extremely simple, only falling droplets of mercury, but you and your collaborators have shown that it can be used for the most diverse purposes.

Several years elapsed before the polarographic method was noticed outside your own country. But after that, its significance has ever increased, not suddenly, attracting the attention of outsiders, but steadily your method has won the confidence of analytical chemists.

On behalf of the Royal Swedish Academy of Sciences I wish to extend to you our warmest congratulations.

May I ask you to advance and receive the Nobel Prize for Chemistry for this year from the hands of our King.

Jaroslav Heyrovský

The trends of polarography

Nobel Lecture, December 11, 1959

The reason why I keep some 38 years to the electrochemical researches with the dropping mercury electrode is its exquisite property as electrode material. Its physical conditions of dropping as well as the chemical changes during the passage of the electric current are well defined, and the phenomena displayed at the dropping mercury electrode proceed with strict reproducibility. Owing to the latter property the processes at the electrode can be exactly expressed mathematically. According to the registering apparatus called « polarograph », which draws automatically curves characteristic of the electrode processes, the electrochemical studies with the dropping mercury electrode and the analytical methods developed on these investigations have been called « polarography ».

The capillary electrode is normally a 8 cm long, 5–6 mm thick tube with the inner bore of 0.05 to 0.1 mm, from which the drops of mercury fall off every 3 to 6 seconds according to the height of the mercury reservoir which is about 40 cm above the tip of the capillary (Fig. 1).

In order that the current passing through this electrode be entirely given by the composition of the solution surrounding it, the second electrode has to be indifferent, unpolarizable, of a constant potential; most suitably it is the layer of mercury at the bottom of the electrolytic vessel (Fig. 2).

To apply the external voltage to the cell we use a potentiometric arrangement shown in Fig. 2. From a 2 or 4 volt lead accumulator an increasing voltage (E) is branched off and the corresponding current is determined by the deflexion of the galvanometer. In such an arrangement the whole applied voltage, E, acts at the small polarizable electrode and determines its potential. The solution has to be conductive by an (about 0.1 N) indifferent electrolyte. Until the year 1924 the galvanometer deflexions were plotted by hand on the ordinate against the voltage applied by pushing the wheel of the Kohlrausch drum to different values. It took a couple of hours to obtain a full graph, point by point from 0 to 2 V. To accelerate the plotting of the curves we have constructed with Shikata in 1924[1] an automatic device, the « polarograph » (Fig. 3) by rotating the Kohlrausch drum mechanically, and syn-

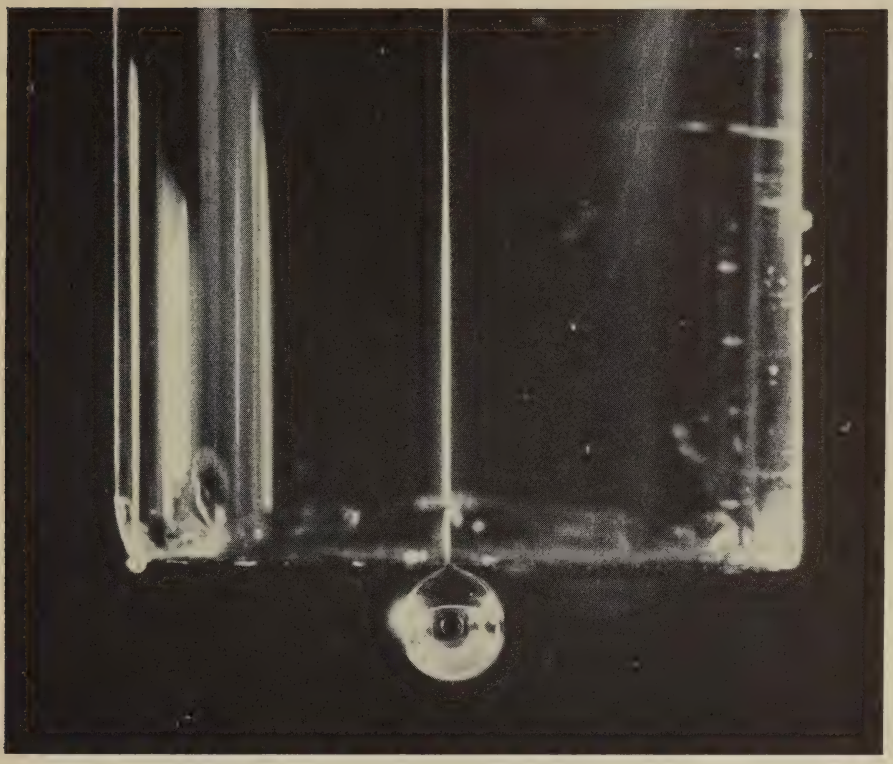

Fig. 1. Mouth of the dropping mercury electrode.

chronously moving also a photographic paper. This mechanism draws the current–voltage diagram (the «polarogram») in less than 10 minutes with all the advantages of automatic recording. The galvanometer has to be damped to register, instead of the instantaneous current, the mean current during each drop. It is not advisable to damp the motion of the galvanometer mirror so far that the oscillations disappear; the regularity of the oscillations is a good check of the right functioning of the apparatus (Fig. 4).

Next, the forms of the current–voltage curves were classified according to the various processes occurring at the dropping electrode. The most fundamental of these is the «charging current» which accompanies every drop-formation conveying to the drop the charge corresponding to the externally applied potential. As this charge depends on the capacity of the dropping electrode and does not involve any electrolytic changes, the current is also termed «capacity current» or «nonfaradayic current» (Fig. 5). It is very small, of the order of 10^{-7} amp pro volt and ordinarily is regarded as

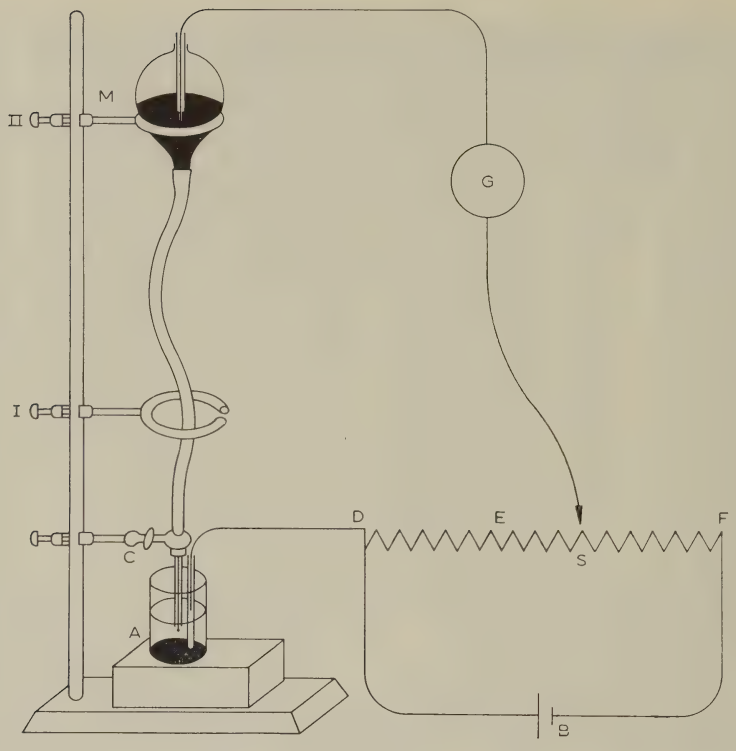

Fig. 2. Simplest polarographic arrangement.

negligible. However, the charging current plays an important role in oscillographic polarography where it is considerably greater. At a certain potential an electrolytic process starts, reduction or oxidation, which is shown by the passage of the current due to electrolysis. In the simplest case molecules or ions of the reducible or oxidizable substances interchange electrons with the electrode and hereby are removed from the electrode surface. Then new molecules of the electroactive substance – the depolarizer – diffuse from the interior of the solution to the surface of the electrode. Ilkovič[2] has calculated the current governed by diffusion to the surface of the expanding drop, deducing (in 1933) the formula of the «diffusion current»:

$$i_d = 0.627\, nF \cdot D^{1/2} m^{2/3} t^{1/6} C$$

where n is the number of electrons involved in the reaction, F the charge of Faraday, D the diffusion constant, m the rate of flow of mercury through the capillary, t the drop-time, and C the concentration of the depolarizer. This

is the most exact electrochemical law announced since Faraday's laws in 1833. When corrected for spherical diffusion according to Koutecký[3], the agreement with experimental results is complete. Fig. 6 gives the reproducibility of a polarogram showing the diffusion current due to atmospheric oxygen in an alkaline solution.

Basing on this law the equations for the current–voltage curves due to the reductions and oxidations of depolarizers were deduced, from which the rule of constancy of the half-wave potentials, $E_{\frac{1}{2}}$, follows[4]. These values introduced new electrochemical constants (Figs. 7, 8) which have their thermodynamic significance giving affinities of electrode reactions. Also constants of complexes can be derived from such equations[5] (Fig. 9).

In 1943 Wiesner[6] discovered another sort of currents controlled by the rate of the chemical reaction yielding the depolarizer at the electrode. For example, formaldehyde or glucose give at ordinary temperature much smaller waves than expected from the Ilkovič formula (Fig. 10). Evidently a

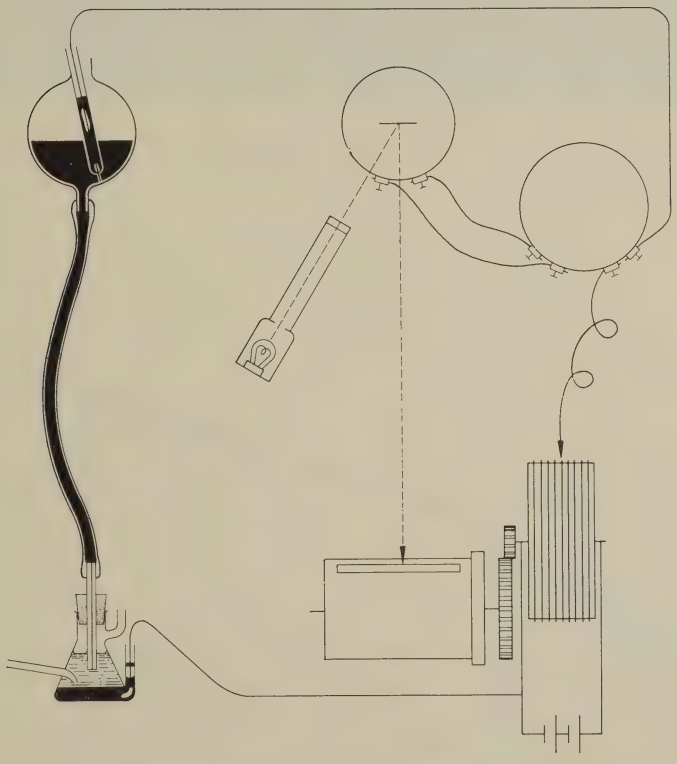

Fig. 3. Scheme of the polarograph.

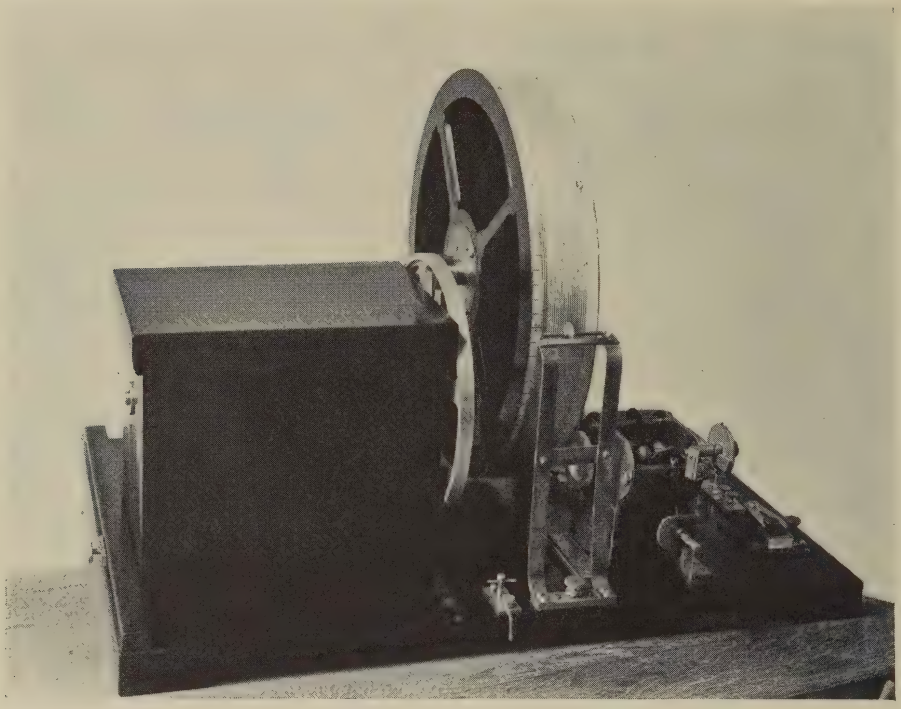

Fig. 4. The first polarograph.

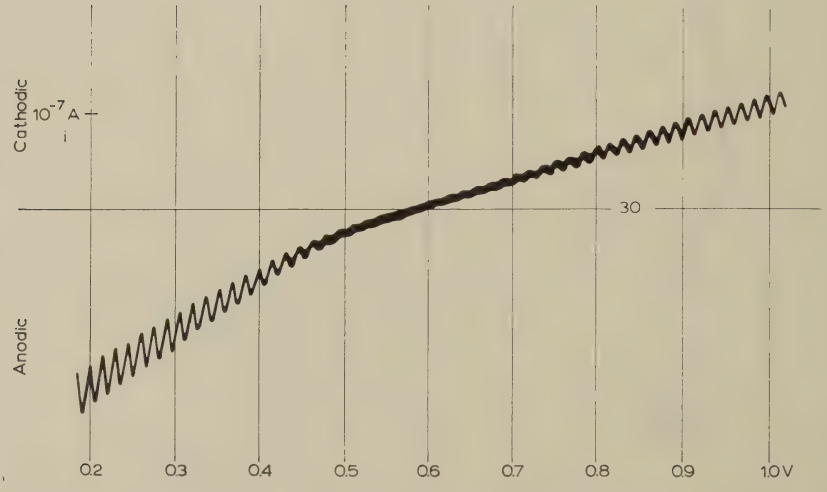

Fig. 5. Capacity current in 0.1 *M* KCl freed from air.

Fig. 6. Diffusion current of oxygen reduction in 0.1 *M* KOH. Curve repeated to show the reproducibility.

slower process than diffusion furnishes the depolarizer. In the case of formaldehyde the molecules of methylene-glycol $H_2C(OH)_2$[7] have to be dehydrated to form the actual depolarizer which is H_2CO. The current is given by the rate of the dehydration, which of course depends on pH. Such currents are termed « kinetic » and bring the possibility to measure the rates of fast chemical reactions. Wiesner *et al.*[8] obtained from the currents due to glucose the kinetic constants of mutarotation and calculated the amount of the free aldehyde in 0.655 *M* glucose at 25° C as 0.0030%. From the ratios of the two waves which appear in the reduction of a reducible acid, we are able to calculate the very high rates of the recombination of the ions with the kinetic constants of the order 10^7 to 10^{11} (Fig. 11).

Contrary to the kinetic currents very much higher are the polarographic currents due to catalytic processes in the evolution of hydrogen, especially those due to SH groups. For example, cystine and proteins in NH_3 buffers containing cobalt ions give a 500 to 700 times higher « catalytic » wave as cystine without the catalyst. Of these reactions the most noteworthy is the serological reaction of Brdička[9] (Fig. 12), to which many publications relate. This is characterized by the « protein double wave » (Fig. 13) which has been found to depend on the pathological state of the patient. All inflammations cause an increase of the protein wave. In non-feverish state it is cancer which is invariably manifested by an elevated protein wave. After the removal of

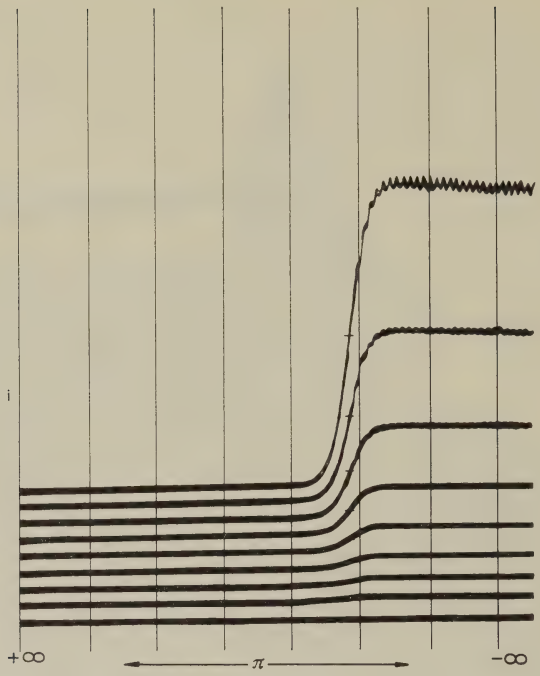

Fig. 7. Constancy of the half-wave potential in solution of various concentrations of $CdCl_2$.

the cancerous centre, the reaction becomes normal; metastases elevate it. It has been ascertained that the proteins responsible for the cancer reaction are mucoproteins, belonging to the γ-2-globulins. Electrophoresis shows that the mucoproteins are akin to the serum component MP-2. On the other hand, liver diseases lower the protein wave, so that in liver troubles the polarographic test is specific.

Another type of polarographic current-voltage curves, closely connected with Kučera's anomalous maximum on the electrocapillary curve, are sharp maxima of current, which at a certain voltage fall to the limiting diffusion current[10] (Fig. 14). An inspection of the dropping electrode through the microscope reveals vehement streaming of the electrolyte along the lines of force to the electrode. Such maxima are very easily suppressed by slight traces of adsorbable matter, like fatty acids, dye-stuffs (Fig. 15), gelatin and other colloids, and in this way indicate very sensitively the degree of purity of solutions. The practical applications of the phenomenon of maxima are manifold. They are termed maxima of the 1st order when formed in dilute electrolytes (Figs. 14, 15). In concentrated solutions there appear rounded

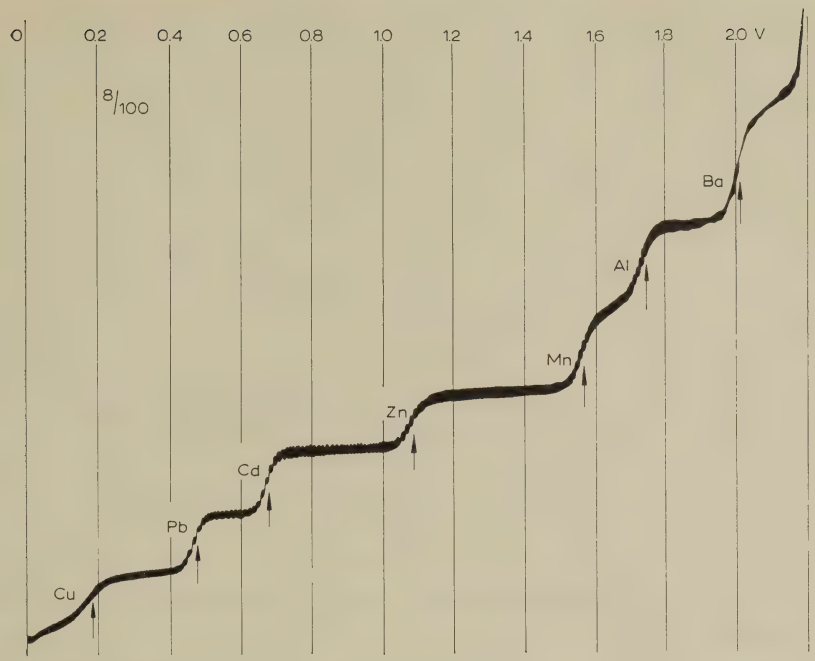

Fig. 8. Polarographic spectrum showing seven cations in the solution.

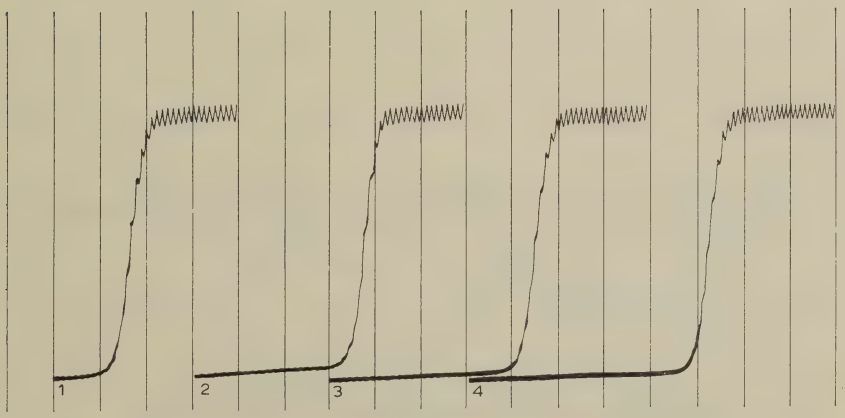

Fig. 9. Shift of the reduction wave of Pb^{2+} ions due to increasing concentration of NaOH. Concentration of lead ions $5.10^{-4}M$, concentration of NaOH (*1* and *2*) $1.2 \cdot 10^{-2}M$; (*3*) 0.1 *M*; (*4*) 1 *M*. Anode potential -0.28 V from S.C.E.

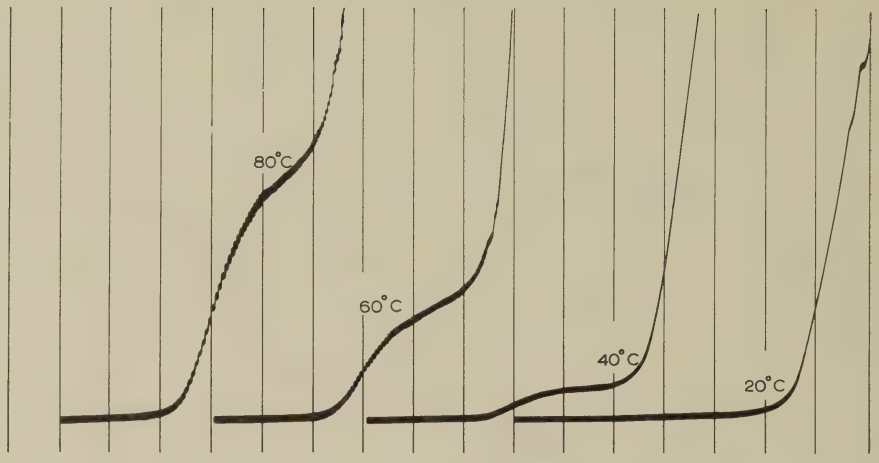

Fig. 10. Kinetic currents of glucose at various temperatures.

maxima of the 2nd order, due to the adhesion of the solution to the moving mercury surface at high rates of flow; they are also suppressed by colloids like gelatin (Fig. 16).

I have only referred to polarography of aqueous solutions. However, similar types of curves and electrode reactions may be followed in many nonaqueous conducting solutions, e.g. in acetonitrile, dioxane, alcohols, glacial acetic acid, sulphuric acid, liquid ammonia, and fused salts. In all of them Ilkovič's law has been found to hold good at all temperatures.

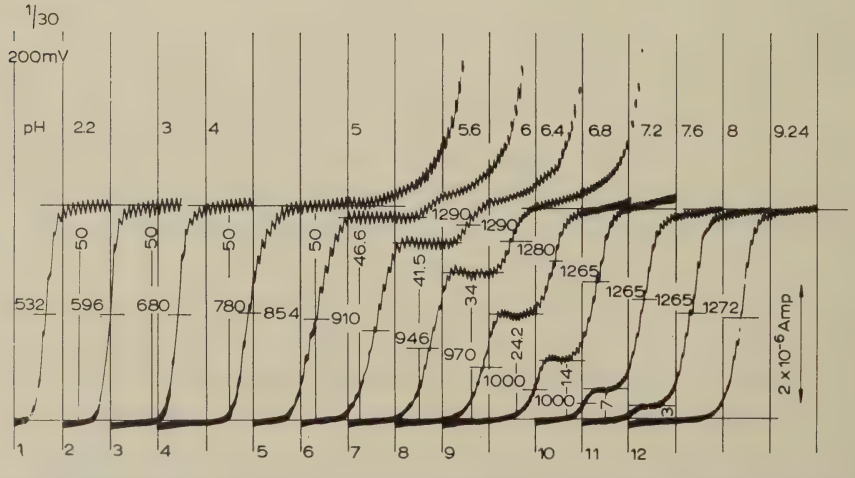

Fig. 11. Polarographic reduction waves of phenylglyoxylic acid in buffer solutions of various pH.

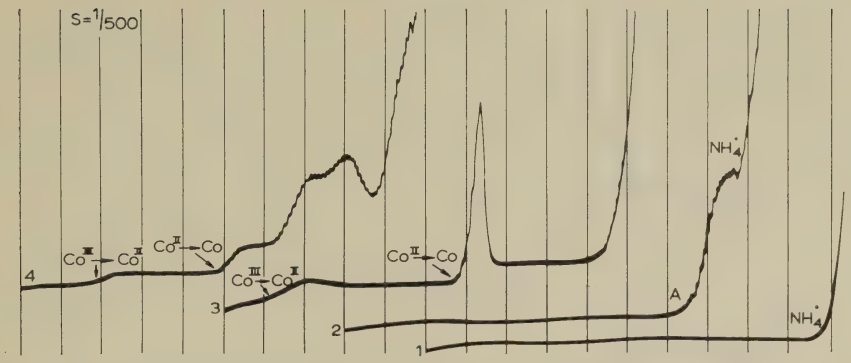

Fig. 12. The effect of proteins in ammoniacal solutions without and with the presence of cobalt.

Curve 1: 0.1 *N* NH_4Cl, 0.1 *N* NH_3 in human serum diluted 1:400. *Curve 2:* in solution. *Curve 3:* 0.001 *M* $Co(NH_3)_6Cl_3$, 0.1 *N* NH_4Cl in human serum diluted 1:400. *Curve 4:* in solution. Curves start at 0 V, abscissae 200 mV.

The current–voltage curves are reproducible enough to yield with sufficient exactness even their derivative curve $di/dE - E$ (Fig. 17). Since at the half-wave potential the ordinary primitive curve has an inflexion, the derivative curve shows there a maximum, the height of which is proportional to the concentration of the depolarizer. The potential of the maximum indicates the quality. There are several methods to obtain the derivative curve automatically.

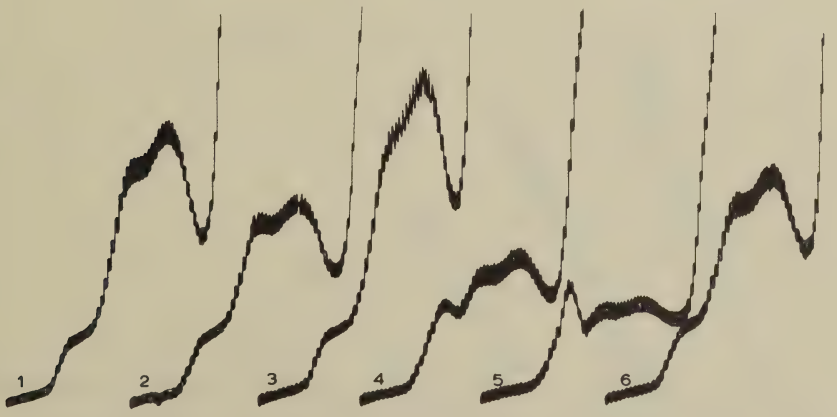

Fig. 13. Brdička's test with blood sera of various pathological states. (1) status febrilis; (2) tumor hepatis susp.; (3) ca ventriculi susp.; (4) normal serum; (5) cirrhosis hepatis; (6) arteriosclerosis.

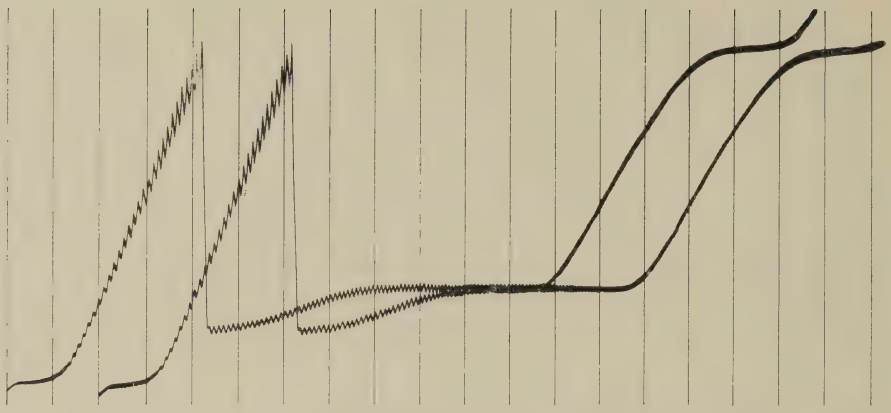

Fig. 14. Maximum of the first order on the wave of oxygen in 0.001 *M* KCl.

The sensitivity of the polarographic method is high, showing depolarizers in a $5 \cdot 10^{-6}$ *M* concentration even in 0.1 cm^3, in a special vessel even in 0.005 mm^3, in which $5 \cdot 10^{-11}$ g of a depolarizer may be ascertained (Fig. 18b). The most sensitive test is that of ruthenium which is shown in a $5 \cdot 10^{-10}$ *M* concentration[12]. With the Barker's «square wave polarograph»[13] where the charging current is practically eliminated, and the electrolytic one strongly amplified, curves are obtained of a derivative character, which show 10^{-8} mol per litre (Fig. 19).

Most sensitive is polarography combined with the measurements of radio-

Fig. 15. Suppression of the maximum by a solution of fuchsin acid salt. (10^{-3} *M* fuchsin acid salt added to 50 ml 0.001 *M* KCl)

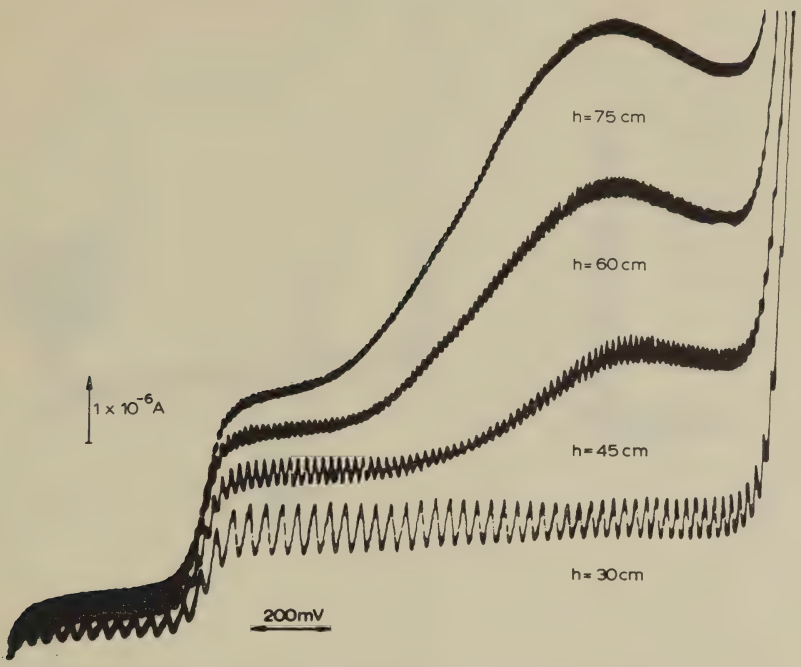

Fig. 16. Maximum of the second order due to $CdCl_2$ in 4.5 *M* KCl. Influence of increasing the rate of flow of mercury (*h*, height of the mercury reservoir). Conditions: 2×10^{-3} *N* $CdCl_2$; N_2 atm.; 18°C.

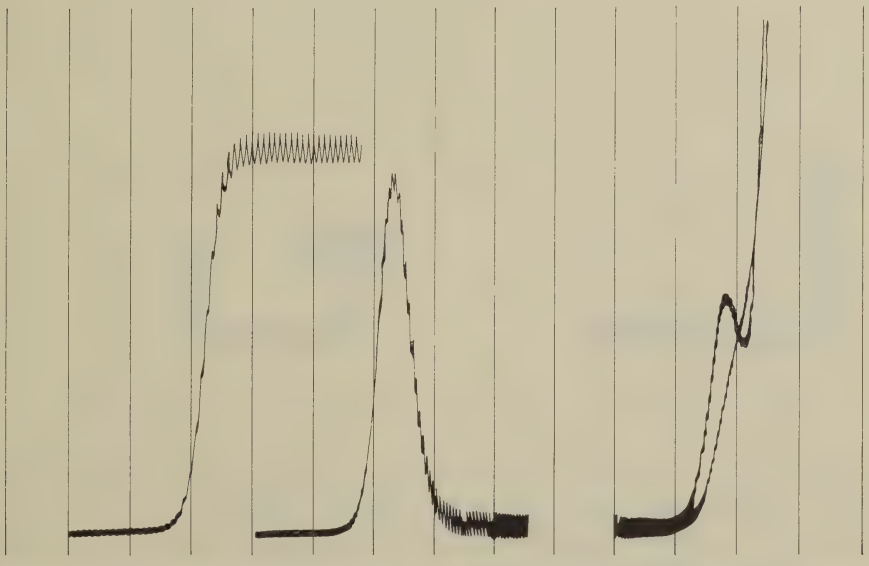

Fig. 17. Primitive and derivative polarographic curves: (*left*) 0.001 *M* $CdCl_2$ in ammonia buffer solution, 0.1 *N* NH_3 in NH_4Cl; (*right*) 0.001 *M* KCl in 0.1 *N* LiOH.

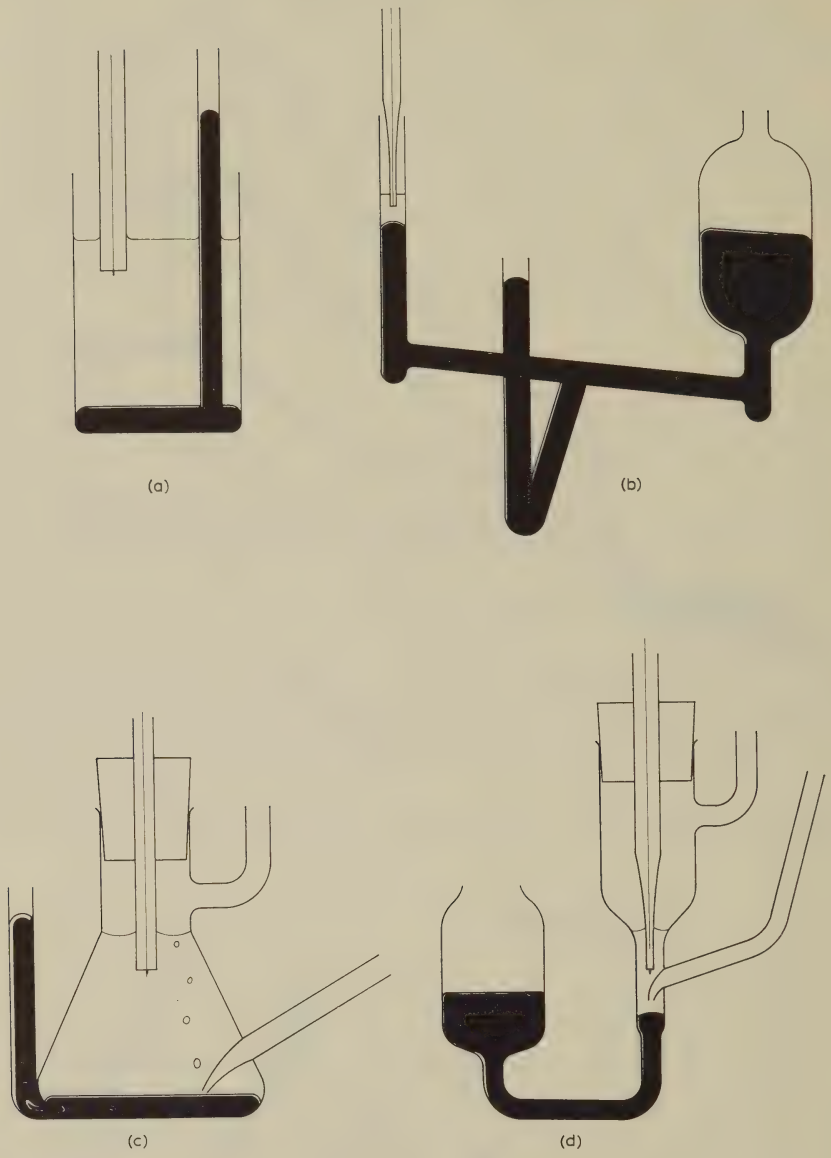

Fig. 18. Polarographic cells for standard- (*a*,*c*) and micro- (*b*,*d*) analysis.

activity (Fig. 20)[14]. The method consists in measuring the activity of the β and γ radiation of mercury drops on which the radioactive metal has been deposited. Isotopes, of course, cannot be distinguished, but e.g. from a mixture of radioactive zirconium 95 and its daughter substance, niobium 95, zirconium 95 can be separated, because it is deposited at the electrode,

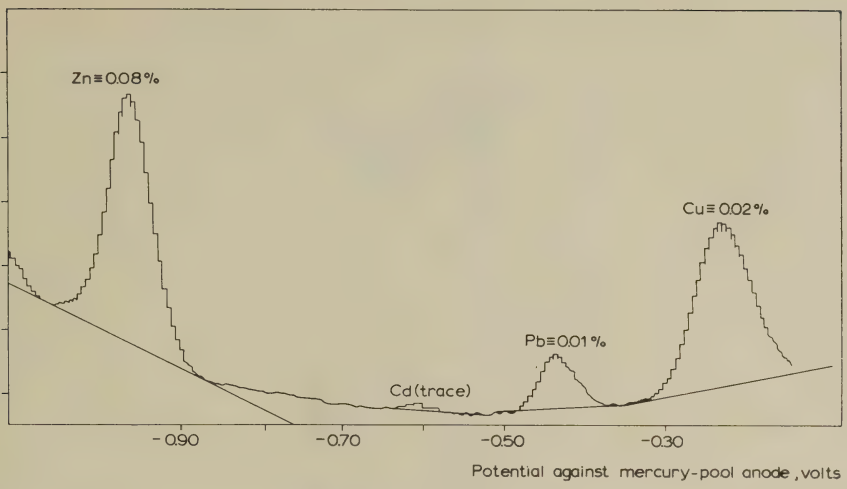

Fig. 19. Square wave polarogram of an analysis of aluminium alloy; 200 mg of sample in 100 ml of 1 *M* KCl.

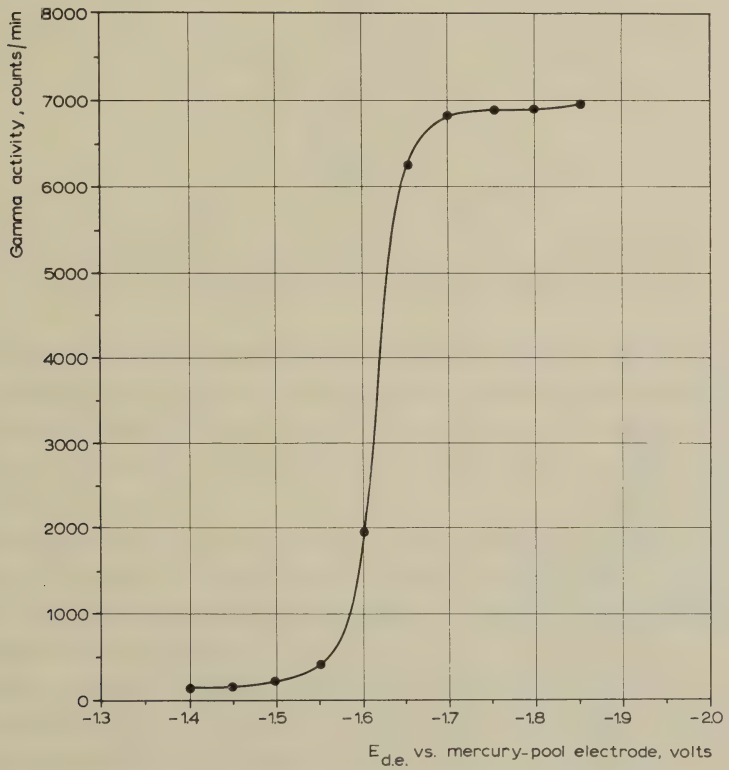

Fig. 20. Plotting of radioactivity of mercury drops against the applied potential.

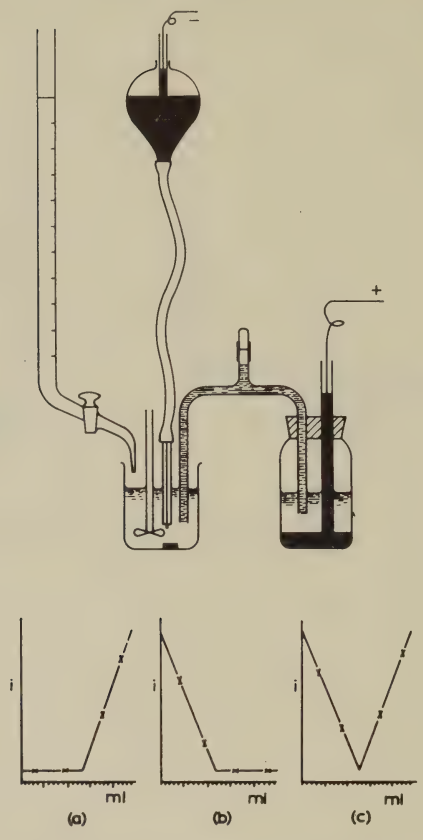

Fig. 21. Limiting current titrations (arrangement and diagrams).

whereas niobium remains in solution. This radioactive technique promises wide applications not only by enabling one to carry out quick, accurate analyses and separations of radionuclides, but also to learn basic electrochemistry of very dilute solutions and electrode reactions. The sensitivity is that of radioactive methods.

All the above-mentioned polarographic methods are used for analytical determinations, qualitative and quantitative, in microdoses as well as macrodoses of inorganic or organic depolarizers. If the substance to be determined is not a depolarizer, it may be chemically converted into a depolarizer and so determined. For example benzene is no depolarizer, its traces in air are noxious and its microdetermination wanted. The smallest traces of benzene are easily converted by nitration into nitrobenzene and after alkalization exactly determined in expired air, in blood or urine.

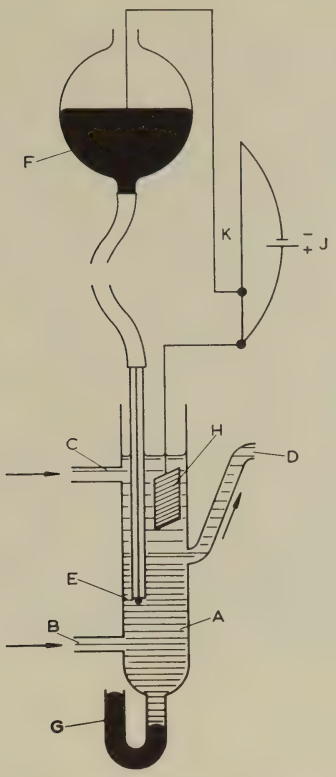

Fig. 22. Scheme of a continuous polarographic analyzer. (E) indicating electrode; (H) redox electrode of constant potential; (A) solution to be analysed; (B) inlet; (C) redox mixture inlet; (D) outlet.

A new trend in volumetric analysis has been introduced by polarographic titrations[15] (1926) later called « polarometric » by Majer (1936) and « amperometric » by Kolthoff (1939), which use the limiting current to indicate the end-point. The indicating electrode may be not only the dropping electrode, but also a rotating or vibrating solid metallic electrode. The accuracy of the polarographic titration here reaches 0.3%. For the titration graph suffice two readings of the current before and two after the equivalence point. The method does not want a polarograph; a potentiometer and a galvanometer of less sensitivity suffice (Fig. 21).

Based on the polarographic experience there are constructed special continuous polarographic analysers[16] following for many weeks without interruption the content of solutions flowing through the cell (Fig. 22). They are used in industry for the automatic control and regulation of technical pro-

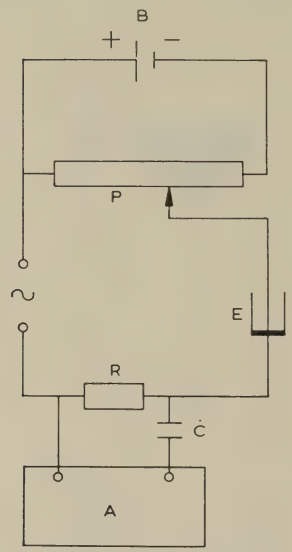

Fig. 23. Circuit of Breyer's alternating-current polarography. (A) voltmeter; (B) accumulator; (C) condensor; (E) dropping mercury cell; (P) potentiometer; (R) resistance; (V) alternating voltage.

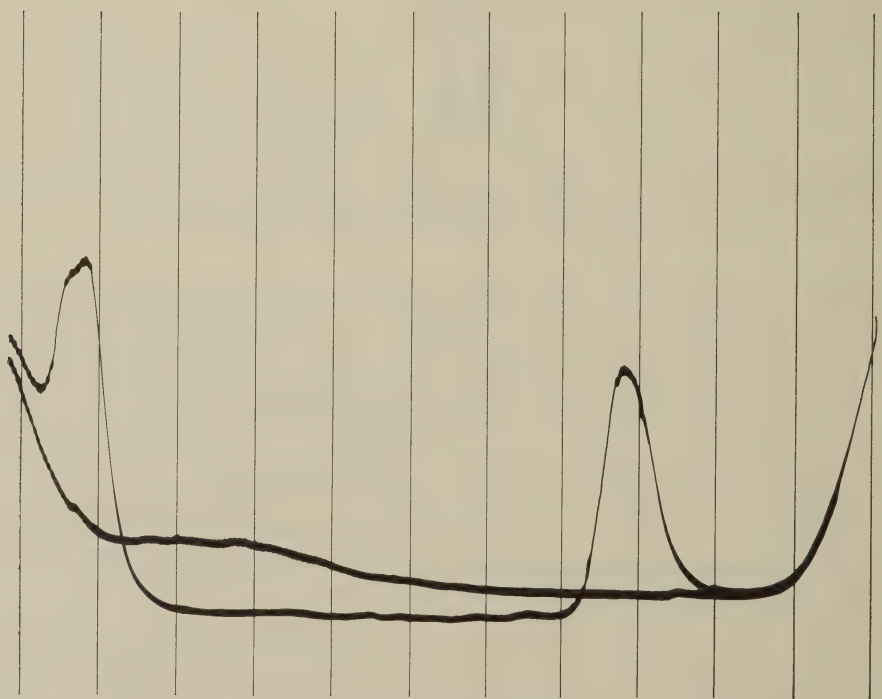

Fig. 24. Tensammetric maxima due to adsorption and desorption of cyclohexanol in acetate buffer solution. (*Upper curve:* pure solution).

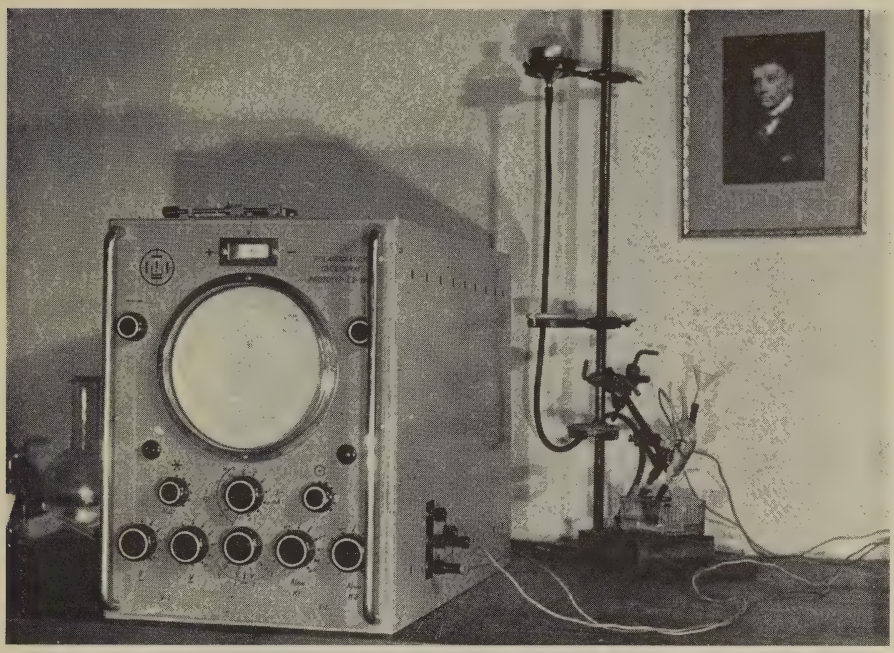

Fig. 25. Oscilloscope for polarographic analysis.

cesses, and for recording the presence of noxious gases in the atmosphere, like SO_2, HCN, CO, etc.

Breyer and his co-workers[17] introduced in 1944 the « tensammetric » method which uses the dropping mercury arrangement by applying a slowly increasing voltage with superposed alternating voltage (Fig. 23) of some 10 to 30 mV and 50 Hz frequency. The alternating current which passes through the polarographic cell is measured, and the current–voltage curve recorded. The method resembles the derivative method inasmuch as it gives peaks at the half-wave potentials of reversible depolarizers, but is very sensitive to adsorbable substances which change the surface tension of mercury electrode (Fig. 25). Hence the name « tensammetry ». The changes of the capacity due to adsorption produce on the current-voltage curves peaks often 100 times higher than is the normal height of the wave of the organic depolarizers. The chief advantage of Breyer's tensammetric method is that it can determine with high sensitivity many organic substances which are polarographically inactive, provided they are surface active.

A great field, opened to polarography since 1938, is « oscillographic polarog-

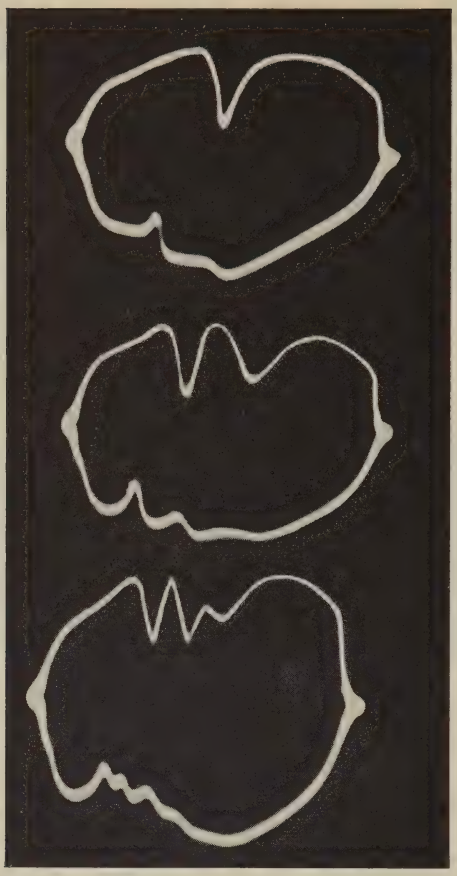

Fig. 26. Oscillographic diagram $dE/dt = f(E)$ of *o*-nitrophenol (*above*), *m*-nitrophenol (*middle*) and mixture of *o*- and *m*-nitrophenols (*below*).

raphy»[18] which substitutes the cathode-ray oscillograph for the galvanometer and voltmeter (Fig. 26). This brings the velocity of recording a polarographic curve to 1/50 second with about the same accuracy of analytical determinations as in classical polarography (Fig. 27).

We meet often with the opinion that polarography did not bring anything new into chemistry except an improvement of analytical methods. That is decidedly not so, since in the study of reductions or oxidations many otherwise inaccessible physico-chemical constants are determinable. Polarography helps the investigation of chemical structure of organic and lately

even inorganic compounds. Striking are the results arrived at by polarographic studies of complexes of Co, Ni, Fe, Cr, the current–voltage curves of which disclose strange valencies. Vlček proved, for example, that cobalt in dipyridyl complexes is reduced[19] from Co^{III} to Co^{II}, and Co^{II} to Co^{I}, and basing on these studies he succeeded in preparing crystalline monovalent

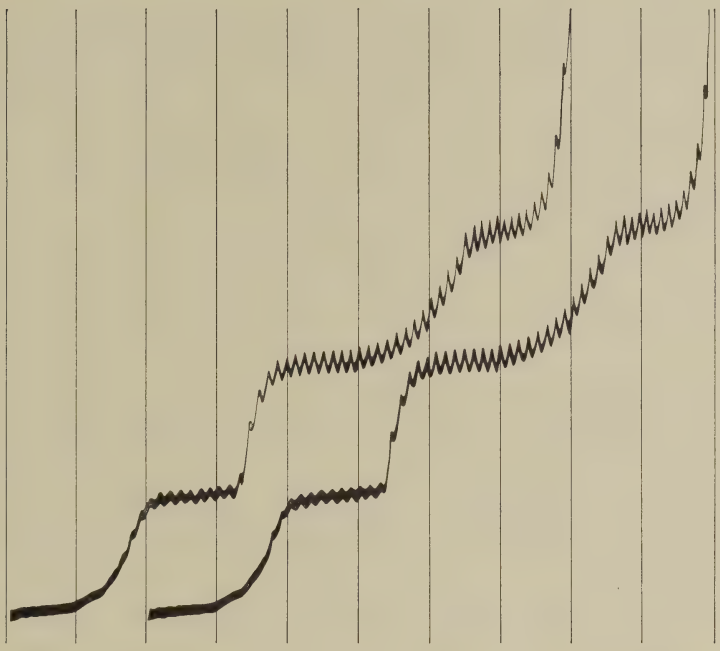

Fig. 27. Three-stage reduction of the chromic tripyridyl complex. Each step corresponds to the uptake of one electron.

$Co(dipyridyl)_2^{I}$ complex. He also proved from polarographic curves the complex compounds of zero-valent Ni, Co, and in the carbonyles negatively divalent Fe and Co. The Cr tripyridyl complex may be successively reduced to Cr^{II}, Cr^{I} and Cr^{0} showing on the polarogram three equal steps (Fig. 27). So far it is the only instance in electrochemistry, in which a trivalent cation can acquire three times one electron in succession.

Although the analytical application of polarography is highly advanced at present, the field of its utilization in basic chemical problems begins to open.

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Biography

Jaroslav Heyrovský was born in Prague on 20th December, 1890, the fifth child of Leopold Heyrovský, Professor of Roman Law at the Czech University of Prague, and his wife Clara, *née* Hanl.

He obtained his early education at secondary school till 1909 when he began his study of chemistry, physics and mathematics at the Czech University, Prague. From 1910 to 1914 he continued his studies at University College, London, under Professors Sir William Ramsay, W. C. Mc. C. Lewis and F. G. Donnan, taking his B.Sc. degree in 1913. He was particularly interested in working with Professor Donnan, on electrochemistry.

During the First World War Heyrovský did his war service in a military hospital as dispensing chemist and radiologist, which enabled him to continue his studies and to take his Ph.D. degree in Prague in 1918 and D.Sc. in London in 1921.

Heyrovský started his university career as assistant to Professor B. Brauner in the Institute of Analytical Chemistry of the Charles University, Prague; he was promoted to Associate Professor in 1922 and in 1926 he became the first Professor of Physical Chemistry at this University.

Heyrovský's invention of the polarographic method dates from 1922 and he concentrated his whole further scientific activity on the development of this new branch of electrochemistry. He formed a school of Czech polarographers in the University, and was himself in the forefront of polarographic research.

In 1950 the Professor was appointed Director of the newly established Polarographic Institute which has been incorporated into the Czechoslovak Academy of Sciences since 1952.

Many universities and seats of learning have honoured Professor Heyrovský. He was elected Fellow of University College, London, in 1927, and received honorary doctorates of the Technical University, Dresden, in 1955, the University of Warsaw in 1956, the University Aix-Marseille in 1959, and the University of Paris in 1960. He was granted honorary membership of the American Academy of Arts and Sciences, Boston, Mass., in 1933; of the

Hungarian Academy of Sciences in 1955; the Indian Academy of Sciences, Bangalore, in 1955; the Polish Academy of Sciences, Warsaw, in 1962; was elected Corresponding Member of the German Academy of Sciences, Berlin, in 1955; member of the German Academy of Natural Scientists, Leopoldina (Halle-Saale) in 1956; Foreign Member of the Royal Danish Academy of Sciences, Copenhagen, in 1962; Vice-President of the International Union of Physics from 1951 to 1957; President and first honorary member of the Polarographic Society, London; honorary member of the Polarographic Society of Japan; honorary member of the Chemical Societies of Czechoslovakia, Austria, Poland, England and India.

In Czechoslovakia he was awarded the State Prize, First Grade, in 1951, and in 1955 the Order of the Czechoslovak Republic.

Heyrovský has lectured on polarography in the United States of America in 1933, the USSR in 1934, England in 1946, Sweden in 1947, the People's Republic of China in 1958, and in U.A.R. (Egypt) in 1960 and 1961.

In 1926 Professor Heyrovský married Marie Koránová, and there are two children of the marriage, a daughter, Judith, and a son, Michael.

Chemistry 1960

WILLARD FRANK LIBBY

«for his method to use carbon-14 for age determination in archaeology, geology, geophysics, and other branches of science»

Chemistry 1960

Presentation Speech by Professor A. Westgren, Chairman of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesty, Your Royal Highnesses, Ladies and Gentlemen.

The Royal Swedish Academy of Sciences this year has decided to award with the Nobel Prize for Chemistry a scientific feat which is less a direct improvement of our material living standards, but more a means of widening and deepening our knowledge in different scientific fields. Professor Willard Libby has been selected to be the prize-winner for his method of age determination of materials of biological origin by use of carbon-14 as a measurer of time. His method has obtained widespread use and has become indispensable in archaeology, geology, geophysics and other sciences. Fortunately, it is so simple – which is probably not always the case with chemical research distinguished with the Nobel Prize – that everyone should be able to understand the conditions and principles for its execution.

Carbon-14 is a kind of carbon, an isotope of carbon with an atomic weight of 14, which is found in the carbon dioxide of the air. It is formed high up in the atmosphere by cosmic radiation coming from outer space. How that occurs, we can ignore. The newly formed carbon-14 has high energy at the moment of its formation, so that it rapidly oxidizes to carbon dioxide, which spreads out and distributes itself evenly in the atmosphere.

The ratio of carbon-14 in the carbon dioxide of the atmosphere is very low. In about one million million of these carbon atoms, there is only *one* which has an atomic weight of 14. But nevertheless, this ratio can be determined, for carbon-14 is a radioactive isotope and manifests itself by its radiation. It is converted into nitrogen by the emission of an electron which can be detected by a sensitive apparatus. The disintegration is such a slow procedure, however, that 5,600 years are required to convert half of these atoms into nitrogen. After another 5,600 years, there is still one quarter left, and after an equally long period of time one eighth, etc. Carbon-14 is thus said to have a half-life of 5,600 years.

If it is assumed that the intensity of the cosmic radiation has been constant during the last few tens of thousands of years, then the average lifetime of carbon-14 – which is approximately 8,000 years – should be sufficiently long

to allow for the formation of a stationary state in the concentration of this isotope not only with reference to the atmosphere, but also to the hydrosphere and biosphere as well. Active and non-active carbon dioxide are dissolved in a contant ratio in the water of the seas and lakes where they are converted into carbonate and bicarbonate, and they are assimilated by trees and plants, and finally also by the animals, which ultimately live on plants. The ratio between active and non-active carbon in all living organisms is the same as that in the air.

When an organism dies, the exchange of carbon with its surroundings ceases and the carbon atoms are inexchangeably held fast in the big molecules of the biological substances. Because the activity of the carbon atoms decreases at a known rate, it should be possible, by measuring the remaining activity, to determine the time elapsed since death, if this occurred during the period between approximatively 500 and 30,000 years ago.

This hypothesis was published by Libby in 1947, and with his great experimental skill it did not take him long to prove the validity of the theory. Recently dead biological substances, such as wood and plant materials, seal oil and others, showed an activity which could be calculated from the knowledge of the production of carbon-14 in the atmosphere, and its rate of decomposition. Fossil material, such as petroleum, was completely inactive; it comes from organisms which lived millions of years ago.

These first control experiments were preceded by a complicated enrichment procedure, but thanks to Libby's experience in working with low-activity substances, he succeeded in refining the activity measurements so that the preliminary concentration procedures became unnecessary. If this had not been possible, his method of age-determination would not have turned out to be the important tool for the advancement of science it has now become.

This refined method was then tested by Libby and co-workers on, among other things, charcoal and wood found in Egyptian graves. The oldest, about 5,000 years old, were from the time of Vizier Hemaka; the youngest, about 2,000 years old, were from the Ptolemaic period, and others were from the time between these two periods. For all of these graves, the Egyptologists had been able to determine the time when they were built. Libby also checked his method by determining the age of heartwood from the trunks of redwood trees (*Sequoia sempervirens*), and of Douglas firs (*Tsuga douglasii*), which were several thousand years old and whose exact age could be determined by counting the annual rings. The results he thus obtained from

these control experiments left no doubts about the reliability of the method.

It was then used to solve problems met with by archaeologists and geologists. Important results were obtained in rapid succession. Egyptologists received important support in their efforts to create a chronology dating back to about two thousand years earlier than the first royal dynasty, which started around 3,400 B.C. It was proved that the last great glacial period in the northern parts of Europe and North America was simultaneous, and still had a considerable extension about 11,000 years ago. Traces of the first human habitations in these regions were dated to about 10,000 years ago. In the southern part of France, on the other hand, beyond the advance of the ice, remnants were found of charcoal from the campfires of human cave-dwellers, remnants which proved to be 15,000 years old. Similar findings were made in Iraq, showing that people lived there 25,000 years ago. This is just to mention a few of the age determinations which throw light on the prehistory of mankind.

It is true that archaeologists and geologists have had methods at their disposal by which they could date their material within the periods of time mentioned here. In Sweden, pollen analysis, and Gerard De Geer's counting of clay layers are well known. The carbon-14 method is a complement to those methods, enabling more accurate determinations to be made.

The carbon-14 method has also been applied in oceanography, for example, for the dating of relatively recent sea sediments. It has made possible more and more accurate determinations of the rate of turnover of the oceanic deep water, and therefore plays an important role nowadays in connection with one of the central problems of physical oceanography, i.e. water circulation in the sea.

Libby's dating method soon attracted attention from the scientific world, and it was not long before carbon-14 laboratories were set up in many countries. Today, some forty institutions carry on investigations in this field, nearly half of them in America. Also here, in Sweden, we have such institutions, and their investigations have given results of great value. All age determinations – nowadays several thousand every year – are published in a general review, and thus made rapidly available throughout the world. The literature in this field has grown from year to year, and at present covers an impressive area.

One of the scientists who suggested Libby as a candidate for the Nobel Prize has characterized his work in the following way: « Seldom has a single discovery in chemistry had such an impact on the thinking in so many fields

of human endeavour. Seldom has a single discovery generated such wide public interest».

Professor Libby. The idea you had 13 years ago of trying to determine the age of biological materials by measuring their carbon-14 activity was a brilliant impulse. Thanks to your great experimental skill, acquired during many years devoted to the study of weakly radioactive substances, you have succeeded in developing a method that is indispensible for research work in many fields and in many institutes throughout the world. Archaeologists, geologists, geophysicists, and other scientists are greatly indebted to you for the valuable support you have given them in their work. The Swedish Academy of Sciences desires to join those who offer you grateful thanks for what you have done for the benefit of so many sciences, and has decided to award you this year's Nobel Prize for Chemistry. May I congratulate you on behalf of the Academy, and ask you to receive the prize from the hands of His Majesty the King.

Willard F. Libby

Radiocarbon dating

Nobel Lecture, December 12, 1960

Introduction

Radiocarbon dating had its origin in a study of the possible effects that cosmic rays might have on the earth and on the earth's atmosphere. We were interested in testing whether any of the various effects which might be predicted could actually be found and used. Initially the problem seemed rather difficult, for ignorance of billion-electron-volt nuclear physics (cosmic ray energies are in this range) was so abysmal at the time and incidentally fourteen years later still is so abysmal, that it is nearly impossible to predict with any certainty the effects of the collisions of the multibillion-volt primary cosmic radiation with air.

Formation of radiocarbon

However, in 1939, just before the war, Professor Serge Korff of New York University and others discovered that the cosmic rays produce secondary neutrons in their initial collisions with the top of the atmosphere. The neutrons were found by sending counters, designed to be sensitive to neutrons, up to high altitudes and they were found to have an intensity which corresponded to about two neutrons being generated for each square centimeter of the earth's surface per second. Whereas it was extremely difficult to predict the types of nuclei that might be produced by the billion-volt primary cosmic rays, the neutrons being secondaries were in the million-volt energy range and therefore subject to laboratory tests. So at this point the question was: « What will million-electron-volt neutrons do if liberated in the air? » The answer to this question was already available – in fact, Professor Korff noted in one of the papers announcing the discovery of the neutrons that the principal way in which the neutrons would disappear would be to form radiocarbon. The reaction involved is a simple one. Oxygen is essentially inert to neutrons but nitrogen is quite reactive. Nitrogen-14, the abundant

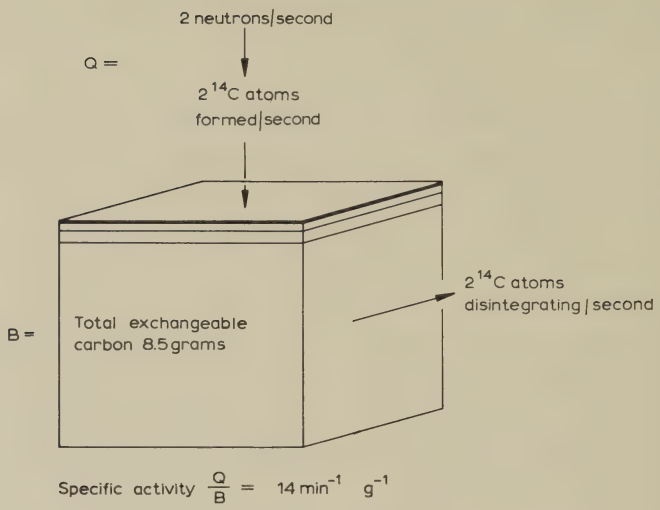

Fig. 1. Radiocarbon genesis and mixing.

nitrogen isotope, reacts essentially quantitatively to form carbon-14 with the elimination of a proton. It also reacts about one percent of the time to produce tritium, radioactive hydrogen, which is another story leading to a method of dating water and wine.

To return to radiocarbon dating – knowing that there are about 2 neutrons formed per square centimeter per second, each of which forms a carbon-14 atom, and assuming that the cosmic rays have been bombarding the atmosphere for a very long time in terms of the lifetime of carbon-14 (carbon-14 has a half-life of about 5,600 years) – we can see that a steady-state condition should have been established, in which the rate of formation of carbon-14 would be equal to the rate at which it disappears to reform nitrogen-14. This allows us to calculate quantitatively how much carbon-14 should exist on earth (see Fig. 1); and since the 2 atoms per second per cm^2 go into a mixing reservoir with about 8.5 grams of carbon per cm^2, this gives an expected specific activity of living matter of 2.0/8.5 disintegrations per second per gram of carbon.

The mixing reservoir consists not only of living matter which dilutes the radiocarbon, but of the dissolved carbonaceous material in the oceans which can exchange carbon with the atmospheric carbon dioxide and thus dilute it. In fact, the ocean is the larger part of the diluting carbon reservoir (see Table 1). For each square centimeter of the earth's surface, there are about 7.25 grams of carbon dissolved in the ocean in the form of carbonate, bicarbonate,

and carbonic acid, and the biosphere itself contains about 0.33 gram per square centimeter of surface. Adding all the elements of the reservoir, we get a total of 8.5 grams of diluting carbon per cm^2, and the 2.0 carbon-14 atoms disintegrating every second should be contained in 8.5 grams of carbon. Thus, the specific activity of living carbon should be that number. We find this to be the actual value observed to within about 10 percent (see Table 2). Of course, the times for mixing of all parts of the reservoir must be short compared to the average lifetime of radiocarbon, 8,000 years. The time for mixing of the oceans is the longest, about 1,000 years on the average.

This is interesting, for it means the following: The present intensity of the cosmic radiation (unless there have been canceling errors in our calculations) corresponds to the average intensity over the last 8,000 years, the average life of carbon-14. It says also that the ocean is mixed nearly perfectly to its bottom depths in 8,000 years. This we know because we included all of the dissolved carbon in the sea. Also, direct measurement of the carbonate and bicarbonate in deep ocean water confirms this. These conclusions could be false if errors in the very different quantities – the intensity of the cosmic rays and the mixing rate and depths of the oceans – should happen just to cancel one another. Being so unrelated, we believe this to be very unlikely and conclude that the agreement between the predicted and observed assays is encouraging evidence that the cosmic rays have indeed remained constant in intensity over many thousands of years and that the mixing time, volume, and composition of the oceans have not changed either.

Table 1. Carbon reservoir make-up (g C/cm^2).

	Anderson and Libby	*W. W. Rubey*
Ocean, «carbonate»	7.25	6.95
Ocean, dissolved organic	0.59	
Biosphere	0.33	0.78
Humus	0.20	
Atmosphere	0.12	0.125
Total	8.5	7.9

We are in the radiocarbon-dating business as soon as this has been said, for it is clear from the set of assumptions that have been given that organic matter, while it is alive, is in equilibrium with the cosmic radiation; that is, all the radiocarbon atoms which disintegrate in our bodies are replaced by the

carbon-14 contained in the food we eat, so that while we are alive we are part of a great pool which contains the cosmic-ray produced radiocarbon. The specific activity is maintained at the level of about 14 disintegrations per minute per gram by the mixing action of the biosphere and hydrosphere. We assimilate cosmic-ray produced carbon-14 atoms at just the rate that the carbon-14 atoms in our bodies disappear to form nitrogen-14. At the time of death, however, the assimilation process stops abruptly. There is no longer any process by which the carbon-14 from the atmosphere can enter our bodies. Therefore, at the time of death the radioactive disintegration process takes over in an uncompensated manner and, according to the law of radioactive decay, after 5,600 years the carbon that is in our bodies while we are alive will show half the specific carbon-14 radioactivity that it shows now. Since we have evidence that this has been true for tens of thousands of years, we should expect to find that a body 5,600 years old would be one-half as radioactive as a present-day living organism. This appears to be true. Measurements of old artifacts of historically known age have shown this to be so within the experimental errors of measurement.

The research on radiocarbon dating consisted of several stages. In the first place, my collaborator, E. C. Anderson, and I had to determine whether the expected radioactivity of living material actually existed. At that time we had no measurement techniques sufficiently sensitive to detect the radioactivities involved directly because they are quite low levels of radioactivity. Later we developed methods for the measurement but at that time we did not have them, so we used the method of concentrating the heavy isotope of carbon. Such an apparatus had been built by and was being used by A. V. Grosse of Temple University, then of the Houdry Process Corporation at Marcus Hook, Pennsylvania. He was concentrating the carbon-13 isotope for medical tracer purposes and kindly agreed to try to concentrate some biological methane for the test so crucial to our research. We had to use biological as contrasted with petroleum methane, for we had at this point arrived at a distinction between living and dead organic chemicals. We had both dead methane and living methane in the sense that methane from oil wells in which the oil has been long buried would be expected to be entirely free from radiocarbon while the methane made from the disintegration of living organic matter should contain 14 dpm (disintegrations per minute) per gram of carbon. The task was to take this living methane and concentrate it in the isotope separation column to see whether the heavy enriched product was radioactive. Happily for our research, it was found to be so and in

about the expected amount. The material used was methane gas from the sewage disposal plant of the City of Baltimore.

The second stage of the research was the development of methods of measurement which were sufficiently sensitive so that we could avoid the use of a ten thousand dollar thermal diffusion isotope column and thousands of dollars of operating expense to measure the age of a single mummy. Obviously, radiocarbon dating would have been an impractical method of measuring archeological ages if this phase of the research had been unsuccessful.

The counting method developed involves measuring the radioactivity of the carbon directly. We convert the samples by chemical methods into a suitable form – carbon dioxide or acetylene gas or even solid carbon – which then is placed inside of a Geiger or proportional counter where it itself constitutes the gas or lies on the inner counter wall. This is possible because carbon as lampblack is an electrical conductor, and the gases CO_2 and C_2H_2 are satisfactory counter gases. In this way a maximum count rate is achieved.

The counter itself is shielded from the background radiations in order to accentuate the carbon-14 count. A typical shield is shown in Fig. 2 (p. 598). It consists of eight inches of iron to absorb the radiations from terrestrial radioactivity, such as uranium, thorium, and potassium. The cosmic rays, however, which consist at sea level largely of μ mesons, penetrate the thick iron shield readily; and whereas the unshielded count rate is about 500 counts per minute, it is decreased to about 100 counts per minute by the iron shield. The 100 remaining counts due in main part to μ mesons have to be removed. In order to do this, we surround the counter with the carbon-dating sample in it with a complete layer of Geiger counters in tangential contact with one another and wire them so that when any one of these counters counts, the central counter with the dating sample is turned off for about one thousandth of a second. In this way the μ mesons are eliminated from the record, so the background comes down to something between 1 and 6 counts per minute, depending on detailed counter and shield design. This is for a counter of about one liter volume capable of holding up to 5 grams of carbon with counting rates of 75 counts per minute for living carbon, 37.5 counts for 5,600-year-old carbon, 18.7 counts for 11,200 years, and 0.07 counts for 56,000 years.

After developing a technique which could measure natural carbon relatively inexpensively with the requisite accuracy, our next job was to determine whether the assumption – that the variation of radiocarbon production due to the variation of the cosmic rays with latitude, which is very strong

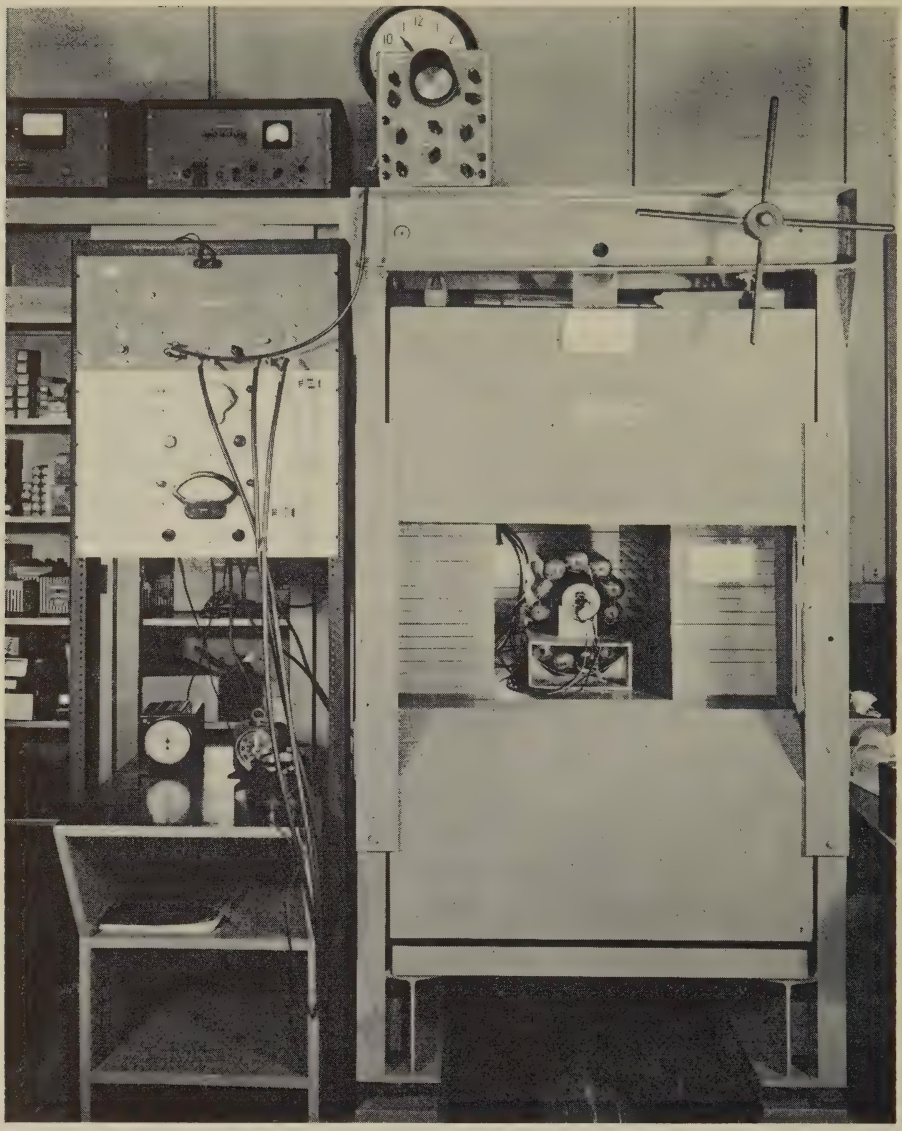

Fig. 2. Radiocarbon counting apparatus.

indeed, would be wiped out by the movement of the winds and the ocean currents in the 8,000-year lifetime of carbon-14 – was sound. The plan was to measure living materials from various places on earth and to see whether they had the same radiocarbon content per gram of carbon. These data on the natural abundance of radiocarbon in the earth were presented by E. C.

Anderson for his doctoral thesis at the University of Chicago. They show no appreciable differences even though they come from places varying in latitude from near the South Pole to near the North Pole (Table 2). At the present time, ten years later, no evidence for variation has been found except in areas of extensive carbonate deposits where the surface waters may carry a considerable amount of old carbon dissolved, and thus reduce the carbon-14 level below the world-wide average for the biosphere–atmosphere–ocean pool as a whole. Fortunately, such conditions are relatively rare and generally easily recognized.

Our whole research was supported generously by the Viking Fund of New York City (now the Axel Wenner-Gren Foundation), the United States Air Force, the Geological Society, the Guggenheim Foundation, and, of course, by the University of Chicago, where most of our research was done.

After the study of the natural occurrence of radiocarbon, the next stage was to see whether we had a method of dating artifacts of a known age. This led us to mummies. J. R. Arnold joined us at this stage. We had a decay

Table 2. Activity of terrestrial biosphere samples.

Source	*Geomagnetic latitude*	*Absolute specific activity (dpm/g)*
White spruce, Yukon	60° N	14.84 ± 0.30
Norwegian spruce, Sweden	55° N	15.37 ± 0.54
Elm wood, Chicago	53° N	14.72 ± 0.54
Fraxinus excelsior, Switzerland	49° N	15.16 ± 0.30
Honeysuckle leaves, Oak Ridge, Tenn.	47° N	14.60 ± 0.30
Pine twigs and needles (12,000 ft. alt.), Mount Wheeler, New Mexico	44° N	15.82 ± 0.47
North African briar	40° N	14.47 ± 0.44
Oak, Sherafut, Palestine	34° N	15.19 ± 0.40
Unidentified wood, Teheran, Iran	28° N	15.57 ± 0.31
Fraxinus mandshurica, Japan	26° N	14.84 ± 0.30
Unidentified wood, Panama	20° N	15.94 ± 0.51
Chlorophora excelsa, Liberia	11° N	15.08 ± 0.34
Sterculia excelsa, Copacabana, Bolivia (9,000 ft. alt.)	1° N	15.47 ± 0.50
Ironwood, Majoro, Marshall Islands	0°	14.53 ± 0.60
Unidentified wood, Ceylon	2° S	15.29 ± 0.67
Beech wood, Tierra del Fuego	45° S	15.37 ± 0.49
Eucalyptus, New South Wales, Australia	45° S	16.31 ± 0.43
Seal oil from seal meat from Antarctic	65° S	15.69 ± 0.30
Average		15.3 ± 0.1

curve drawn which predicted with no unknown factors, no adjustable constants, the specific activity of ancient organic matter. And so the question was to see whether it worked. The first thing we had to do, of course, was to get the materials for measurement. This was done by enlisting the cooperation of the American Anthropological Association and the American Geological Society. Geologists have been quite interested in the results of this dating technique from the beginning even though its reach in time is short for many of their problems. A committee of advisors consisting of Donald Collier, Richard Foster Flint, Frederick Johnson, and Froelich Rainey was appointed to select the samples for us and to help us collect them. These distinguished gentlemen worked hard for several years, assisting and collecting the samples and advising us. The research in the development of the dating technique consisted of two stages – the historical and the prehistorical epochs. The first shock Dr. Arnold and I had was when our advisors informed us that history extended back only to 5,000 years. We had thought initially that we would be able to get samples all along the curve back to 30,000 years, put the points in, and then our work would be finished. You read statements in books that such and such a society or archeological site is 20,000 years old. We learned rather abruptly that these numbers, these ancient ages, are not known accurately; in fact, it is at about the time of the First Dynasty in Egypt that the first historical date of any real certainty has been established. So we had, in the initial stages, the opportunity to check against knowns, principally Egyptian artifacts, and in the second stage we had to go into the great wilderness of prehistory to see whether there were elements of internal consistency which would lead one to believe that the method was sound or not.

For the prehistoric period our committee set up a network of problems which were designed to check, in as many ways as possible, points of internal consistency. They set out about a dozen major projects, and we collected samples from each of these projects and worked hard and measured them, and similar measurements are still going on now ten years later.

Fig. 3 shows the Curve of Knowns – the results obtained for samples of known age compared to the carbon-14 decay curve drawn from the present living matter value of 14 taken as unity, with a half-life of 5,568 $\pm$ 30 years. The half-life itself was measured in 1949 in collaboration with A. G. Engelkemeir, W. H. Hamill, and M. G. Inghram to be 5,580 $\pm$ 45 years, a value which when combined with independent values of 5,589 $\pm$ 75 by W. M. Jones, and 5,513 $\pm$ 165 by W.W. Miller, R. Ballentine, W. Bernstein, L.

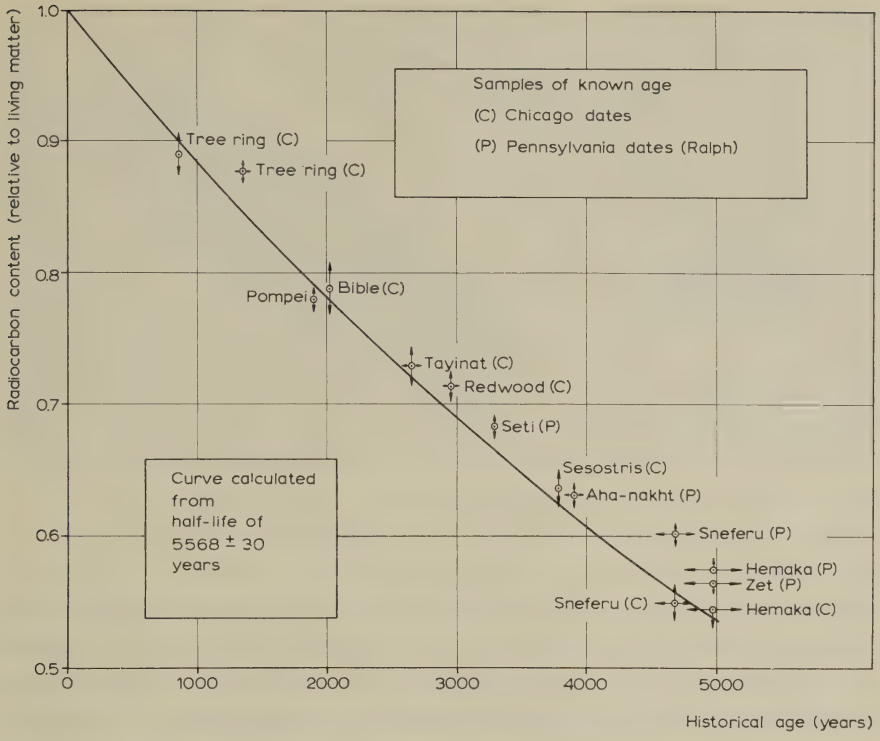

Fig. 3. Curve of Knowns.

Friedman, A. O. Nier, and R. D. Evans, gave 5,568 ± 30 by weighting according to the inverse square of the errors quoted. Re-measurements are in progress now by Mann at the National Bureau of Standards in Washington and by Olsson in Uppsala.

The knowns are in two main groups – those measured by us at the University of Chicago and those measured by Miss Ralph at the University of Pennsylvania labeled (C) and (P) respectively. One sample « Pompeii » was measured by E. A. Olson and W. S. Broecker of the Lamont Geological Observatory.

The oldest samples of known age measured were Hemaka and Zet from the First Dynasty in Egypt. Both were wood found in the subterranean brick structures of the First Dynasty tombs of the Vizier Hemaka and of King Zet, both at Saqqara. Hemaka was contemporaneous with King Udimu, and both tombs were generally agreed to be 4,900 ± 200 years before the present. The next oldest samples were cedar wood from the upper chamber of the Southern Pyramid of Sneferu at Dahshur. The next sample marked Sesostris is a very interesting one. It is a part of the deck of the funeral ship

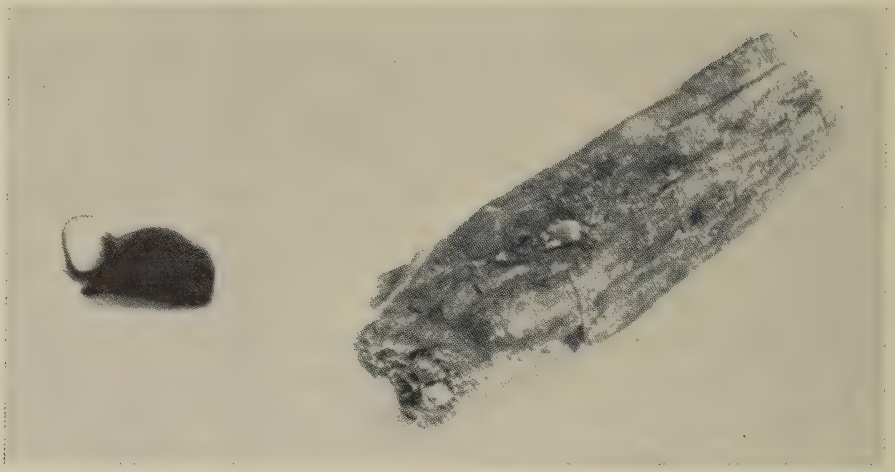

Fig. 4. Hair of Egyptian woman, 5,000 years old.
Wood from glacial moraine, more than 20,000 years old.

which was placed in the tomb of Sesostris III of Egypt and is now in the Chicago Museum of Natural History. It is about 20 feet long, 6 feet wide, and is quite an imposing object, complete with paddles. Our sample came from the deck. The next sample is Aha-nakht. It consisted of wood, probably cedar from the outer sarcophagus of Aha-nakht, at El Bersheh. It was found in the tomb which was covered with earth. The coffin was presumably excavated by the natives at the same time as the El Bersheh coffin obtained for the British Museum by E. A. W. Budge after 1895.

Proceeding up the curve, the next sample is the heartwood of one of the largest redwood trees ever cut. The tree was known as the « Centennia Stump » felled in 1874 with 2,905 rings between the innermost (and 2,802 rings between the outermost) portion of the sample and the outside of the tree. Therefore, the known mean age, according to the tree-ring method of Douglas was 2,928 $\pm$ 51 years, as of the time it was cut. This is an interesting point, as it shows that in the heartwood of the *Sequoia gigantea* at least the sap is not in chemical equilibrium with the cellulose and other large molecules of the tree. In other words, the carbon in the central wood was deposited there about 3,000 years ago, although the tree itself was cut just a few years ago. The next sample, which is marked Tayinat, is from a house in Asia Minor which was burned in 675 B.C. It was wood from the floor of a central room in a large Hilani (« Palace ») of the « Syro-Hittite » period in the city of Tayinat in Northwest Persia. Its known age is 2,625 $\pm$ 50 years.

The next is the linen wrapping of one of the Dead Sea Scrolls, the Book of Isaiah, which was found in Palestine a few years ago. The next sample, labeled «Pompeii», was carbonized bread from a house of ancient Pompeii still retaining the appearance of a baker's roll that seemingly was overdone, which was charred by the volcanic ashes that buried the city in 79 A.D., roughly 1880 years ago. The other samples are wood which were dated by the Douglas tree-ring counting technique. Taking these samples all together, the agreement with the predicted radiocarbon content seems to be satisfactory. The errors are given as the counting errors (standard deviations) only.

It is certainly possible that the decay curve, which is drawn according to the half-life of 5,568 years, could be drawn somewhat differently. However, it is well to know that all radiocarbon dates published today have been calculated on this half-life; and in order to avoid confusion, we should be careful in changing the basis of the calculation of radiocarbon ages until the evidence for a change in half-life is definite. The Curve of Knowns seems to indicate that a slightly longer half-life might be permissible. However, there are other possible explanations of a deviation of the Curve of Knowns from the theoretical curve. We will all await the results of the half-life researches of Dr. Mann and Miss Olsson with great interest.

It has been observed that fossil carbon dioxide from the combustion of coal and oil beginning at about 1870 began to dilute the biosphere and to reduce the radiocarbon content until 1954, when the explosion of atomic devices reversed the trend. The carbon-14 introduced by the neutrons produced in the explosions more than compensated for the reduction by the fossil carbon, which at that time had amounted to about 3 percent in the Northern Hemisphere as compared to the primeval level extending as far back as has been

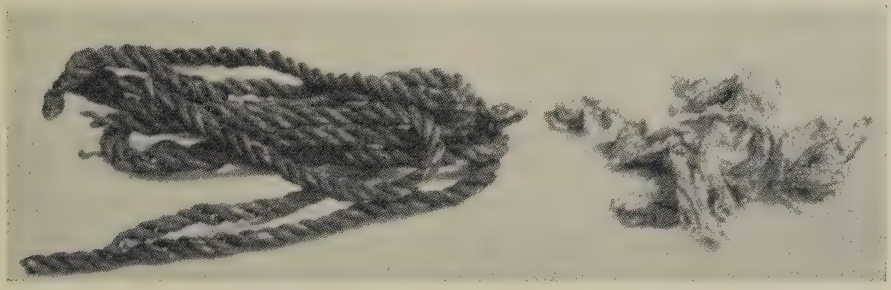

Fig. 5. Peruvian rope, 2,600 years old.
Linen wrappings of Book of Isaiah of Dead Sea Scrolls, 2,000 years old.

possible to measure by tree rings. Hl. de Vries and Hans E. Suess have been particularly active in research on this point. Dr. Suess, in fact, discovered the fact that fossil carbon dioxide was reducing this specific activity in recent biospheric material as compared to the general level prior to 1870. Broecker and Olson have made careful studies of the carbon-14 content of ancient woods as well. And the general result is that prior to 1870 there appeared to be only very minor variations on the order of one percent or less in the radiocarbon content of living matter. The recent perturbations are of no great concern for present living archeologists and geologists. Of course, in the future it will be difficult to correct for the period when these perturbations were active; i.e., 5,000 years from now there may be some difficulty in understanding why for the period of a century or so, beginning in 1870, the radiocarbon level was so perturbed. However, the written records may well explain the anomaly. And, in fact, radiocarbon dating as such may not be needed to establish historical fact.

Following the test of the Curve of Knowns, the next step in the research was to test in the great periods of prehistory to see whether the dates obtained were reasonable. Perhaps the most interesting single general result in this prehistoric period is the time at which the last ice sheet moved down to cover the northern part of the United States and the European continent. The result 11,400 years $\pm$ 200 years has now been well established by the radiocarbon technique; i.e. the radiocarbon dates for this cataclysmic development show first that it happened simultaneously in Europe and in North America and that the phenomenon was very widespread, and that it had a tremendous impact on the living habits of the people the world over; e.g., the oldest sign of man in Northern Europe and in England is younger than this, presumably because of the thoroughness with which the glacier removes all sorts of human artifacts. So the oldest of the Scandinavian and English occupation sites, all are about 10,400 years, corresponding presumably to the time when the ice sheet receded.

In Fig. 6, we have plotted for the Americas just the number of occupation sites versus age measured by radiocarbon. It is quite clear that there is an abrupt discontinuity at about the time of 10,400 years. In Europe, however, if instead of examining sites in northern regions we look at Mediterranean basin sites, there is no discontinuity, and evidences of human occupation extend back as far as the radiocarbon dating technique can reach – 50,000 years or so. There seems to be some contrast between this and the situation in the Americas, where, as shown in Fig. 6, one sees a decided difference in

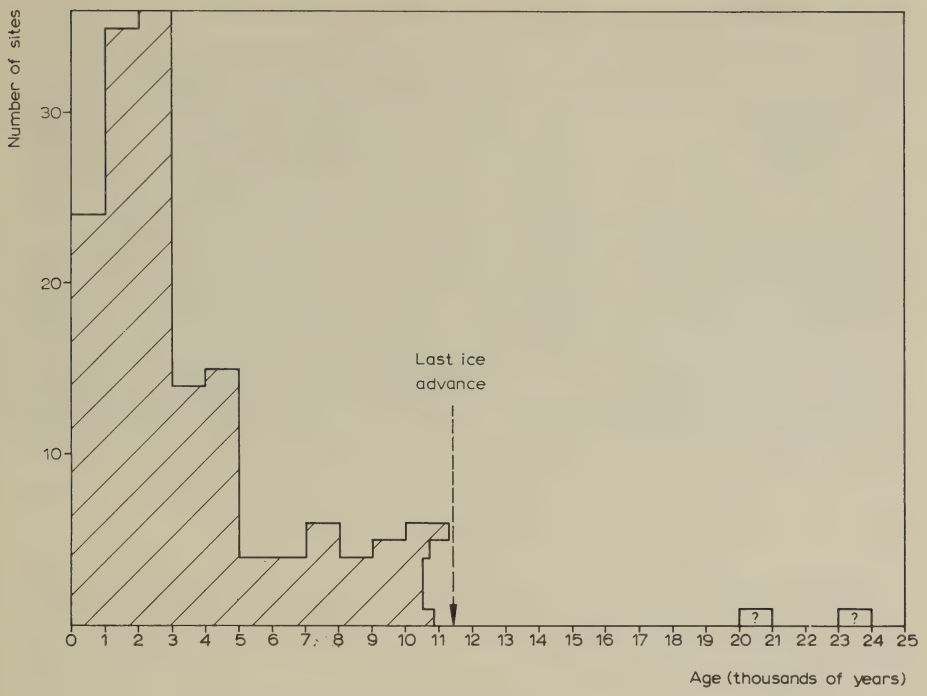

Fig. 6. Number of human sites in the Americas *vs.* age.

the total number of sites in preglacial times. In view of the fact that it is known that extensive areas of the Americas were not glaciated by the last ice sheet, this raises something of a question. There is, of course, the definite possibility that this is pure accident, and it even seems possible that we do now have human sites in the Americas which are definitely older than 10,400 years. However, just the weight of the evidence seems to indicate that something in the way of a discontinuity occurred at that time. Most of the sites that are older than 10,400 years are equivocal in one way or another, at least to the chemist or physicist who overhears the archeologists arguing about them. We have noticed that there is a considerable unanimity of opinion about American sites of 10,400 years or younger being human sites; whereas, there is considerable tendency for discussion and debate for the older sites. This is not true, however, in Southern Europe and Asia Minor. One of the most remarkable of the sites in Europe is the Lascaux Cave in Central France, which has the beautiful paintings on the walls, showing the ancient animals in such authentic style as to demonstrate the remarkable advancement of the culture of the people at that time. These paintings are presumably older than

15,000 years because the charcoal found in the soil of the cave had this value. Around Asia Minor and in the areas of the Middle East, there is no scarcity of materials which go back as far as radiocarbon can reach, with considerable evidence that the sites are human in nature.

In addition to the work on human history, radiocarbon has been used for geological purposes to a considerable extent. Of course, the time span of radiocarbon is so short compared to the history of the earth, that most geological problems are outside its reach. But recent history and recent events do fall within its grasp, and there have been a number of investigations, particularly the sorting out and measuring of the chronological events of the recent ice ages; i.e., the relative times of arrival of the various ice advances and the periods of time between them and the points of simultaneity, and the identification of particular moraines with particular advances. On these points, small as they are, and perhaps relatively unimportant, the geologists have found radiocarbon to be of some use.

In oceanography, the great question of the rate of mixing of the oceans has yielded to the radiocarbon technique to a considerable extent, particularly in the hands of Suess and Broecker and Olson; Suess particularly in the Pacific, and Olson and Broecker in the Atlantic. They have shown that the Pacific mixes relatively less rapidly, the turnover time being something between 1,500 and 2,000 years; whereas, the Atlantic mixes relatively more rapidly at a rate about twice this, or with a 750- to 1,000-year turnover time. It is clear from these researches that the fundamental assumption of radiocarbon dating, that the reservoir of the sea must be counted as a diluent for the cosmic ray carbon-14, is valid. Further, it has been shown by Suess that there will be opportunities of measuring the deep ocean currents. He finds evidence for velocities and directions of the deep ocean currents in the Pacific corresponding to some hundreds of years for the passage northward along the bottom.

In meteorology, radiocarbon dating has had some usefulness. It has been interesting to observe the changes of the radiocarbon content in living matter near large industrial centers where the rate of production of carbon dioxide from coal and oil was highest, and also to observe the dissemination of the radioactive carbon made by atomic explosions in the atmosphere. From these things we know that there is world-wide mixing which occurs. We observe the effects which generate very largely in the Northern Hemisphere quite clearly in the Southern Hemisphere, though they are reduced somewhat in intensity. This is the first time that there has been clear and incontrovertible

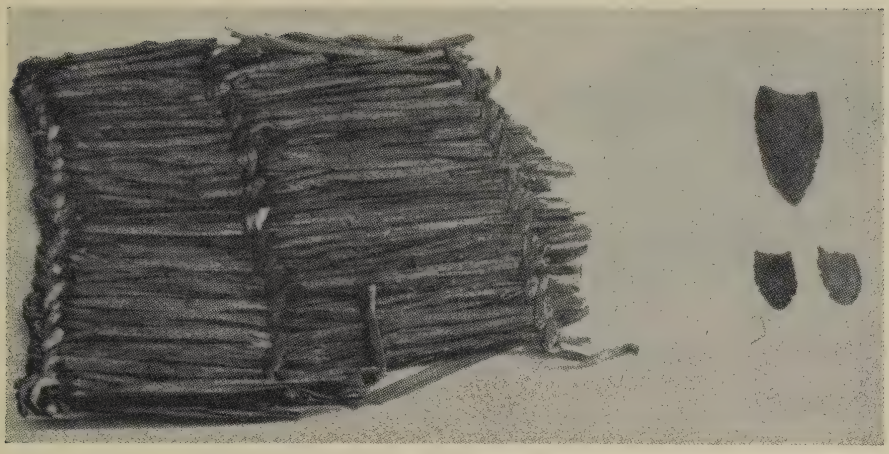

Fig. 7. Rush matting from Nevada cave, 3,000 years old.
North American arrowhead (replicas), 10,000 years old.

evidence for such a world-wide circulation and on a time scale of a matter of a very few years, particularly in the case of the bomb-test carbon-14.

Of course, the main point of radiocarbon dating is archeology and the investigation of the history of man through the use of chemistry, for most of the ancient men did not write and we have no written records except in Egypt, Asia Minor, and in limited areas of Central America. Yet it is perfectly clear that back 10,000 and more years ago, people lived so as to rival modern man in intelligence and capabilities. We have just to look at their handiwork to see this. The paintings in the Lascaux Cave, the handiwork of the ancient Indians in North America – particularly the basketry and the very skilfully made arrowheads – attest to their great capabilities. Where they came from perhaps we do not know, but we do know that they were very intelligent and very capable people.

Last spring, on an island off the coast of California, Santa Rosa Island, friends of mine found a 6-foot skeleton, 10,400 years old by the radiocarbon measurements of Dr. Broecker of Lamont Geological Observatory on some charcoal found next to the skeleton. This is the same 10,400 years date which we have observed so often and now marks the early sign of man in Santa Rosa Island; the Lindenmeier Site in Colorado; the Clovis Site; the Lamus Cave in eastern Nevada on the Utah-Nevada border, where continuous occupancy occurred from the time of the melting of the last glacier 10,400 years ago down to the time when modern man came into the area; the Fort

Fig. 8. Rope sandal found in Oregon Cave, 9,000 years old.
Dung of extinct ground sloth, 10,000 years old.

Rock Cave in Oregon, where the most beautiful basketry of ancient man was discovered – grass rope woven into sandals of beautiful shape and design – three hundred pairs of them neatly stacked just as though they were a community store of shoes 9,000 years old; and several other sites in the Americas. We see in this the evidence that man has been a long time learning to write history, but has been making history for many thousands and perhaps tens of thousands of years.

In Central Europe the element of simultaneity, which is revealed by the radiocarbon dates for the people who did not write or leave records, establishes conflicts and clashes between cultures which are interesting to examine and speculate upon. The Neanderthal man and Cro-Magnon man did not stay long together. The Neanderthal man disappeared and the Cro-Magnon man won – he may have been the man who painted the beautiful Lascaux Cave paintings, as I understand it from the archeologists.

We learn various details about the ancient peoples. For example, in the time of Hammurabi, the Babylonian King, there was an accurate calendar, but we were not certain about the correlation of this calendar with our own. It was a very good calendar the Babylonians had, but there is an uncertainty which, as I understand it, is due to the identification of a particular eclipse as we calculate it backwards in time in order to arrive at the correlation to our own calendar. Therefore, careful measurements were made on a portion of a house about 4,000 years old that was accurately dated on the Babylonian calendar. In this case, a serious attempt was made to test the limit of sensitivity of the radiocarbon dating method. The sample of wood came from a beam

from the roof of a house in Nippur which bore a clear and legible Hammurabian calendar date. The beam was divided into three equal portions which were carefully measured with a total measurement time of three months and the results on the three portions were then coordinated to arrive at a definite answer as to which of the most likely correlations of the Christian Calendar with the Babylonian Calendar was correct. We concluded that the younger of the possible calendars was strongly favored and that the odds against the other being correct were something like 9 to 1.

With the advancement of the radiocarbon dating technique and the consequent increase in accuracy, at least of the relative dates, it is possible to do more of these difficult jobs of pinpointing past events in time so as to drive back history into prehistoric periods and to more clearly delineate what really did happen in the development of man. The determination of the chronology of ancient civilizations may be said to be the main archeological problem and task of radiocarbon dating. As the technique is developed further and more fully and is more widely used, it should be possible to excavate and utilize sites which are now hardly more than dark spots in some remote area. Charcoal is one of the best materials for radiocarbon dating, providing adequate care is taken that intrusive rootlets and humic acids are removed before measurement.

We intend at UCLA to attempt to make a portable radiocarbon dater which will allow us to work in the field with the archeologists and geologists in obtaining dates which, though not as accurate as those which would be obtained in the laboratory, may be useful enough to serve as guides during the digging. The problem is to find a truck which will carry the rather heavy equipment over the rough country which is usually involved. If this effort is successful, it will be a development which will bring the carbon daters and the archeologists and geologists even closer together. Of all the rewards of research, none is greater than the meeting of people in different fields and the finding of interests in common. It will be most refreshing and rewarding for the radiocarbon daters to go out and partake, at least vicariously, in the great thrill of an archeological dig.

The large number of people who have contributed to the development of the radiocarbon dating technique, several of whom I have mentioned today, but many of whom I have been unable to mention, are largely responsible for whatever success it has had. We now have several thousand radiocarbon dates throughout the fields of archeology, geology, meteorology, oceanography, and other areas. From the examination of the results, it is possible to

form an opinion as to the general reliability and general weaknesses of the method. I am sure that Dr. Arnold would agree with me in saying that it has lived up to our fondest hopes. It was clear from the beginning that there would be difficulties about the samples. Anyone knows it is possible to get dirt into solid matter which is lying in the ground, even if it is there only for a brief period, let alone many thousands or tens of thousands of years. The saving aspect of the situation, however, is that it is very much more difficult to mix molecules in such a way that they cannot be separated chemically, particularly in the case of substances such as charcoal and wood and cloth, and even in certain cases for limestone and shale. One can separate and distinguish the contaminant from the original material and in this way disclose the real radiocarbon content. The researches of a number of people have validated the assumption that it is possible and that, indeed, it is not too difficult to obtain authentic samples in the field. In general, the samples may have to be inspected with some care under a relatively high-powered glass and then possibly treated with properly chosen chemicals. But all of these things can be done and with techniques that are no more difficult than those used by the average hospital technician, and a sample can be obtained which should give authentic radiocarbon dates. The dating technique itself is one which requires care, but which can be carried out by adequately trained personnel who are sufficiently serious-minded about it. It is something like the discipline of surgery – cleanliness, care, seriousness, and practice. With these things it is possible to obtain radiocarbon dates which are consistent and which may indeed help roll back the pages of history and reveal to mankind something more about his ancestors, and in this way perhaps about his future.

W. F. Libby, *Radiocarbon Dating*, 2nd ed., Univ. of Chicago Press, Chicago, Ill., 1955.
R. F. Flint and E. S. Deevey, Jr., (Eds.), *American Journal of Science, Radiocarbon Supplement*, Vol. I (1959), 218 pp.
R. F. Flint, E. S. Deevey, Jr., and Elizabeth G. Weinman, (Eds.), *American Journal of Science, Radiocarbon Supplement*, Vol. II (1960), 228 pp.

Biography

Willard Frank Libby was born in Grand Valley, Colorado, on 17th December, 1908, to Ora Edward Libby and his wife Eva May (*née* Rivers).

He attended grammar and high schools near Sebastopol, California, between 1913 and 1926, moving to the University of California at Berkeley in 1927, where he studied till 1933, taking his B.Sc. and Ph.D. degrees in 1931 and 1933 respectively. He was appointed Instructor in the Department of Chemistry at California University (Berkeley) in 1933 and during the next ten years was promoted successively to Assistant and then Associate Professor of Chemistry. He was awarded a Guggenheim Memorial Foundation Fellowship in 1941 and elected to work at Princeton University, but on 8th December, 1941, this Fellowship was interrupted for war work on America's entry into World War II, and Libby went to Columbia University on the Manhattan District Project, on leave from the Department of Chemistry, California University, till 1945.

At the end of the war, in 1945, Libby accepted the post of Professor of Chemistry in the Department of Chemistry and Institute for Nuclear Studies (now the Enrico Fermi Institute for Nuclear Studies) of Chicago University, remaining there till his appointment by President Eisenhower on 1st October, 1954, as a member of the U.S. Atomic Energy Commission.

This appointment was renewed by the President for a further five-year term on 19th June, 1956, but Libby resigned from it on 30th June, 1959, to become Professor of Chemistry in the University of California at Los Angeles, being appointed Director of the Institute of Geophysics and Planetary Physics on 1st January, 1962.

Libby has performed a wide range of scientific advisory and technical consultant work with industrial firms associated with the Institute for Nuclear Studies, as well as with defence departments, scientific organizations and universities. From 1945 to 1952 he was a Member of the Committee of Senior Reviewers of the Atomic Energy Commission; from 1950 to 1954 he was a Member of the Commission's General Advisory Committee and was re-appointed to this Committee by President Eisenhower in June, 1960.

He has been a Member of the Plowshare Advisory Committee since 1959; a Guggenheim Memorial Foundation Fellow in 1941, 1951 and 1959–1962; a Member of the Advisory Board of the Guggenheim Memorial Foundation, being re-elected in May, 1960, for a further term of four years; a Research Associate of the Carnegie Institute of Washington Geophysical Laboratory from 1954 to 1959. He has served as Consultant to the Office of Civil and Defence Mobilization (1959–1961) and also as a Member of the Advisory Council to the Coordinator of Atomic Activities of the State of California since 1959. He is, since 1963, Director of the Douglas Aircraft Company, and Member of the National Science Foundation's General Commission on Science, the Federal Government and the Academic Institution.

Libby is a Physical Chemist, and specialist in radiochemistry, particularly hot atom chemistry, tracer techniques, and isotope tracer work. He became well-known at Chicago University for his work on natural carbon-14 (radiocarbon) and its use in dating archaeological artifacts, and natural tritium, and its use in hydrology and geophysics.

Besides the Nobel Prize in Chemistry for 1960, he received other distinctions, including the Research Corporation Award for 1951 for the radiocarbon dating technique; the Chandler Medal of Columbia University for outstanding achievement in the field of chemistry (1954); the American Chemical Society Award for Nuclear Applications in Chemistry (1956); the Elliott Cresson Medal of the Franklin Institute (1957); the American Chemical Society's Willard Gibbs Medal Award (1958); the Albert Einstein Medal Award (1959); the Day Medal of the Geological Society of America (1961).

Libby's book, *Radiocarbon Dating*, was published by the University of Chicago Press in 1952, and a second edition appeared in 1955. He is also the author of numerous articles, which appeared principally in scientific journals. Since 1960 he is member of the Editorial Board of the *Proceedings of the National Academy of Sciences*, and since 1962 member of the Editorial Board of *Science*.

Professor Libby holds memberships of numerous learned societies in the United States; he is also Member of the Heidelberg Academy of Sciences, of the Bolivian Society of Anthropology, and is Foreign Member of the Royal Swedish Academy of Sciences (1960).

He is married to the former Leonor Hickey of King City, California. They have twin daughters: Janet and Susan (b. 1945).

Chemistry 1961

MELVIN CALVIN

«for his research on the carbon dioxide assimilation in plants»

Chemistry 1961

Presentation Speech by Professor K. Myrbäck, member of the Nobel Committee for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Your Royal Highnesses, Ladies and Gentlemen.

In order to grow and to perform its various activities, every living organism needs a supply of energy in some suitable form. In this respect the organisms existing on this planet can be divided into two fundamentally different groups. All animals, including man, and also some lower organisms, require a supply of energy-rich organic material, food-stuffs that « contain calories », to use a popular expression. The energy contained in the food-stuffs is made available by a biological oxidation (« combustion ») of carbohydrates, fats, etc. Obviously, these types of organisms, the so-called heterotrophic organisms, are absolutely dependent on supplies of organic material, occurring outside themselves.

As opposed to the heterotrophic organisms, the organisms belonging to the second group, the so-called autotrophic organisms, i.e. the green plants and certain bacteria, do not require organic material supplied from without. They synthesize organic compounds, primarily carbohydrates, from simple substances, carbon dioxide and water, substances that, in themselves, do not contain any calories. The energy needed for the synthesis is supplied by light which is absorbed by the organisms and subsequently converted by them from light energy into chemical energy. The sequence of reactions by which carbon dioxide and water are converted to carbohydrate is called carbon dioxide assimilation or, taking into account the role of light energy, photosynthesis.

It becomes obvious that photosynthesis not only provides an explanation for the existence of the autotrophic organisms but also furnishes food for man and animals. In other words, photosynthesis is the absolute prerequisite for all life on earth and the most fundamental of all biochemical reactions. It has been estimated that plants and microorganisms on earth transform about 6,000 tons of carbon from carbon dioxide to carbohydrate per second, with at least four-fifths of this amount contributed by organisms in the oceans.

It is understandable that a reaction of such importance and such dimensions should attract the interest of science at an early stage. For more than a

century, however, progress in the understanding of the chemistry of photosynthesis was very slow, partly for want of suitable experimental methods.

More than fifty years ago it was recognized that photosynthesis comprised two distinct phases, light reactions and dark reactions. The Nobel Laureate today, Dr. Melvin Calvin, has spent many years of research work on the chemistry of both phases of photosynthesis and, in the case of the second phase, that is to say the reactions leading from carbon dioxide to the assimilation products – to quote Calvin, « the path of carbon in photosynthesis » – his work has resulted in the complete clarification of an extremely intricate problem.

Success was achieved as a result of sharp-witted, skilful and persistent work, to some degree facilitated by the availability of certain modern experimental methods that allow investigations which, in older times, were simply impossible. Two such methods may be mentioned: the method of the isotopic labeling of molecules, introduced by de Hevesy, and the chromatographic methods, developed by Martin and Synge, which permit the separation of minute quantities of compounds in complicated mixtures. By an ingenious combination of these and many other methods, Calvin succeeded in tracking the path of the carbon atom from carbon dioxide, taken up by the plant, to the finished assimilation products. The radioactive carbon isotope, ^{14}C, well-known also in other connections, has played a particularly important role in Calvin's work.

Most of Calvin's experiments have been performed using a microscopic green alga, *Chlorella pyrenoidosa*, but parallel experiments with higher plants have shown that the mechanism of carbon dioxide assimilation is the same in all plants.

A question that had occupied scientists for more than a century, was: « What is the primary product of the assimilation; what first happens to the carbon dioxide taken up by the plant? » Calvin demonstrated that the primary reaction is not, as had been assumed previously, a reduction of carbon dioxide as such, but a fixation of carbon dioxide to a substance, the carbon dioxide acceptor, occurring in the plant. Calvin was able to show that the product formed in this fixation reaction is an organic compound known as phosphoglyceric acid.

This discovery was of fundamental importance for the development that followed. The primary product of assimilation was recognized as being a compound, well-known from earlier work as an intermediary product of the biological degradation of carbohydrates, and not some previously un-

known compound; phosphoglyceric acid had been identified as a breakdown product of sugar as early as 1929 by Ragnar Nilsson here in Stockholm. Calvin's identification of the primary assimilation product with phosphoglyceric acid led to the very important conclusion that there is an intimate connection between photosynthesis and carbohydrate metabolism as a whole.

Calvin's subsequent investigations mapped out the path between the primary product and the end products of assimilation, the various carbohydrates. What had formerly been assumed to be a reduction of carbon dioxide was shown to be a reduction of phosphoglyceric acid. For a reduction of phosphoglyceric acid to the carbohydrate level, the plant has to supply both a reducing agent and a so-called energy-rich phosphate. It is for the production of these co-factors that plants utilize light energy. This means that light energy is not directly involved in the reactions of assimilation; light energy is used for regeneration of co-factors which are consumed in the assimilation reactions.

As mentioned above, the primary reaction in the assimilation is a fixation of carbon dioxide to an acceptor, the chemical nature of which has been established by Calvin. Rather unexpectedly, this acceptor was found to be a derivative of a sugar, ribulose, to which nobody had paid much attention previously. When carbon dioxide is fixed to the ribulose derivative, phosphoglyceric acid is formed.

As the acceptor is consumed during the fixation reaction it must obviously be regenerated from the assimilation products. Calvin has elucidated the very complicated mechanism of this regeneration. Between the primary product and the acceptor there are no less than ten intermediary products and the reactions between these products are catalyzed by eleven different enzymes.

Professor Melvin Calvin. Your investigations on plant photosynthesis have shed light on a field of biochemistry which was, until recently, veiled in obscurity. You have tracked the various steps of the path of carbon in photosynthesis and created a clear picture of this complicated sequence of reactions, reactions of immense importance for life on our planet.

On behalf of the Royal Academy of Sciences, I extend to you our warm congratulations, and I ask you to receive this year's Nobel Prize for Chemistry from the hands of His Majesty the King.

Melvin Calvin

The path of carbon in photosynthesis

Nobel Lecture, December 11, 1961

Introduction

It is almost sixty years since Emil Fischer was describing on a platform such as this one, some of the work which led to the basic knowledge of the structure of glucose and its relatives[1]. Today we will be concerned with a description of the experiments which have led to a knowledge of the principal reactions by which those carbohydrate structures are created by photosynthetic organisms from carbon dioxide and water, using the energy of light.

The speculations on the way in which carbohydrate was built from carbon dioxide began not long after the recognition of the basic reaction and were carried forward first by Justus von Liebig and then by Adolf von Baeyer and, finally, by Richard Willstätter and Arthur Stoll into this century. Actually, the route by which animal organisms performed the reverse reaction, that is, the combustion of carbohydrate to carbon dioxide and water with the utilization of the energy resulting from this combination, turned out to be the first one to be successfully mapped, primarily by Otto Meyerhof[2] and Hans Krebs[3].

Our own interest in the basic process of solar energy conversion by green plants, which is represented by the overall reaction

$$CO_2 + H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} (CH_2O)_n + O_2$$

began some time in the years between 1935 and 1937, during my postdoctoral studies with Professor Michael Polanyi at Manchester. It was there I first became conscious of the remarkable properties of coordinated metal compounds, particularly metalloporphyrins as represented by heme and chlorophyll. A study was begun at that time, which is still continuing, on the electronic behavior of such metalloporphyrins. It was extended and gen-

eralized by the stimulus of Professor Gilbert N. Lewis upon my arrival in Berkeley. I hope these continuing studies may one day contribute to our understanding of the precise way in which chlorophyll and its relatives accomplish the primary quantum conversion into chemical potential which is used to drive the carbohydrate synthesis reaction.

Even before 1940 the idea that the reduction of carbon dioxide to carbohydrate might be a dark reaction separate from the primary quantum conversion act was already extant, stemming most immediately from the comparative biochemical studies of Cornelis van Niel and the much earlier work of F. F. Blackman and its interpretation by Otto Warburg. The photo-induced production of molecular oxygen had been separated chemically and physically from the reduction of carbon dioxide by the demonstration of oxygen evolution by illuminated chloroplasts. This was done by Robert Hill[4] using ferric iron as oxidant in the place of carbon dioxide.

We are thus able to represent in a diagrammatic way (Fig. 1) the overall conversion of light energy into chemical energy in the form of carbohydrate and oxygen. The light energy first absorbed by chlorophyll and related pigments is converted into chemical potential in the form of high-energy containing compounds, represented by B in Fig. 1. These, in turn, lead to the production of oxygen from water and the simultaneous generation of high-level reducing agents which can be used, together with whatever collaborators are required, to carry out the carbon dioxide reduction.

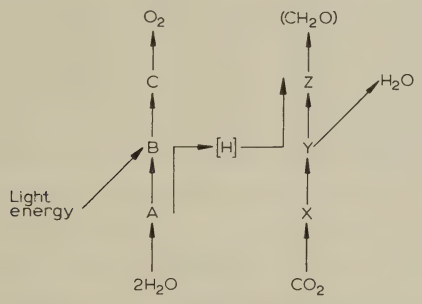

Fig. 1. Elementary photosynthesis scheme.

One of the principal difficulties in such an investigation as this, in which the machinery which converts the CO_2 to carbohydrate and the substrate upon which it operates are made with the same atoms, namely, carbon and its near relatives, is that ordinary analytical methods will not allow us to

distinguish easily between the machinery and its substrate. However, the discovery of the long-lived isotope of carbon, carbon-14, by Samuel Ruben and Martin Kamen[5] in 1940 provided the ideal tool for the tracing of the route along which carbon dioxide travels on its way to carbohydrate, represented in Fig. 1 by the series of unknown materials, X, Y, Z, etc.

In 1945 it became apparent to us that carbon-14 would be available cheaply and in large amounts by virtue of the nuclear reactors which had been constructed. With the encouragement and support of Professor Ernest O. Lawrence, the Director of the Radiation Laboratory in Berkeley, we undertook to study that part of the energy-converting process of photosynthesis represented by the carbon-reduction sequence, making use of ^{14}C as our principal tool.

Design of the experiment

The principle of the experiment was simple. We knew that ultimately the CO_2 which enters the plant appears in all of the plant materials but primarily, and in the first instance, in carbohydrate. It was our intention to shorten the time of travel to such an extent that we might be able to discern the path of carbon from carbon dioxide to carbohydrate as the radioactivity which enters with the CO_2 passes through the successive compounds on its way to carbohydrate.

Preliminary experiments confirmed the idea that the absorption of CO_2 and its incorporation in organic material was indeed a dark reaction. This was easily established by exposing plants which had first been illuminated in the absence of carbon dioxide so as to store some of the intermediate high-energy containing compounds, and then noting that these compounds could be used in the dark to incorporate relatively large amounts of CO_2. However, the products did not proceed very far along the reduction scheme under these conditions, and so we undertook to do the experiment in what we call a steady state of photosynthesis.

Plant material

As the precision of our experiments increased, the need for more reproducible biological material also increased, and very soon we found it necessary to grow our own plant material in as highly reproducible manner as

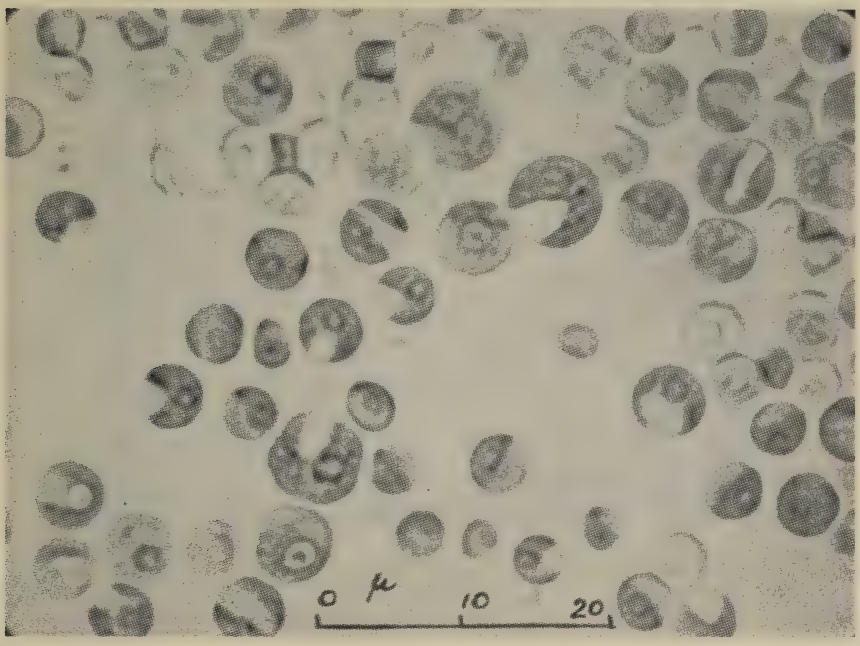

Fig. 2. Photomicrograph of *Chlorella*.

possible. A very convenient green plant that had already been the subject of much photosynthetic research was the unicellular green alga, *Chlorella*, a photomicrograph of which is shown in Fig. 2. We developed methods of growing these organisms in a highly reproducible fashion, both in intermittent and continuous cultures, and it is with organisms such as these that most of our work was done. I hasten to add, however, that the essential features of the cycle with which we finally emerged were demonstrated on a wide variety of photosynthetic organisms, ranging from bacteria to the higher plants.

Apparatus

The exposures were initially performed in a simple apparatus (called a «Lollipop» because of its shape, Fig. 3) which contained a suspension of the algae undergoing photosynthesis with normal CO_2. The initiation of our tracer experiment was accomplished by injecting into the non-radioactive carbon dioxide stream, or substitution for it, some ^{14}C-labeled CO_2 for a suitable period of time, ranging from fractions of a second to many minutes. At the end of the preselected time period, the organisms were killed by va-

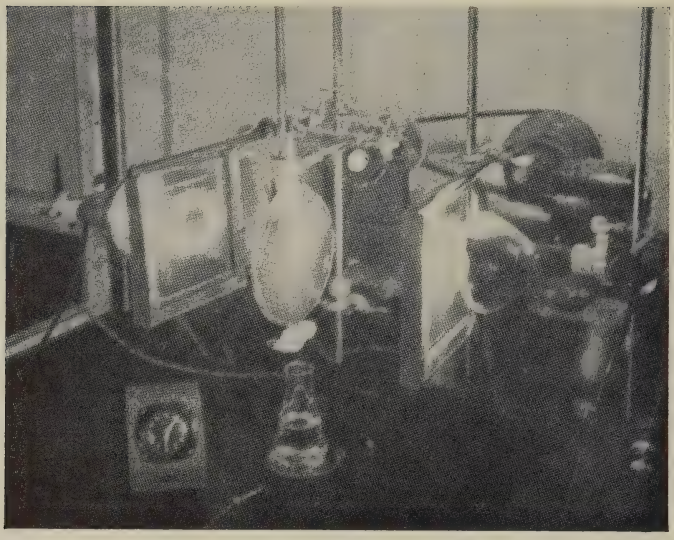

Fig. 3. «Lollipop», the apparatus in which the photosynthesis experiments are performed.

rious methods, but principally by dropping the suspension into approximately four volumes of alcohol. This stopped the enzymic reactions and, at the same time, began the extraction of the materials for analysis.

Early analytical methods

In the early work, the classical methods of organic chemistry were applied in our isolation and identification procedures, but it soon became apparent that these were much too slow and would require extremely large amounts of plant material to provide us with the identification of specific labeled compounds. Here, again, we were able to call upon our experience during the war years in which we had used ion exchange columns for the separation of plutonium and other radioactive elements. We made use of both anion and cation exchange columns, and soon discovered that the principal compounds in which we were interested, that is, those which became ^{14}C radioactive in the shorter exposure times, were, indeed, anionic in character.

Because of the peculiar difficulty we found in eluting the principal radioactive components from anion exchange resins, it became apparent that this radioactive material was a strongly acidic material and very likely had more than one anionic point of attachment to bind to the resin. Among these

peculiarities was the fact that an ordinary carboxylic acid could be eluted relatively easily while the principal radioactive material would require either very strong acid or very strong base to bring it off. This, taken together with a number of other chromatographic properties, led to the idea that these early products might very well be phosphate esters as well as carboxylic acids.

A more detailed analysis of the precise conditions required to elute the material off the ion exchange columns suggested phosphoglyceric acid as a possibility. To a relatively large amount of algae was added as indicator a small amount of the purified radioactive material obtained from a small sample of algae exposed to radioactive carbon for a few seconds. This led to the direct isolation of slightly over nine milligrams of a barium salt which by classical organic procedures we were able to show to be the barium salt of 3-phosphoglyceric acid[6].

Paper chromatographic methods

About this time Martin and Synge[7] had developed their method of partition chromatography which was particularly well adapted for amino acid analysis because of the sensitivity of the colorimetric detection method. We turned to this as our principal analytical tool. It was particularly suited to our needs because, having spread our unknown material from the plant onto a sheet of filter paper by two-dimensional chromatography, we could then find the particular components which we sought, namely, the radioactive ones, without knowing their chemical nature beforehand. This was done by placing the paper in contact with photographic film, thus exposing the film at those points of the paper upon which were located the very compounds in which we were interested.

The result of such an experiment in which the algae were exposed to radioactive CO_2 for thirty seconds under what we then thought were steady state conditions is shown in Fig. 4. The blackened areas on the film indicate the presence of radioactive compounds on the paper at those points. Such a chromatogram and film as shown in Fig. 4 constituted our principal primary source of information.

It should be noted that this information resides in the number, position and intensity, that is, radioactivity of the blackened areas. The paper ordinarily does not print out the names of these compounds, unfortunately, and our principal chore for the succeeding ten years was to properly label those blackened areas on the film.

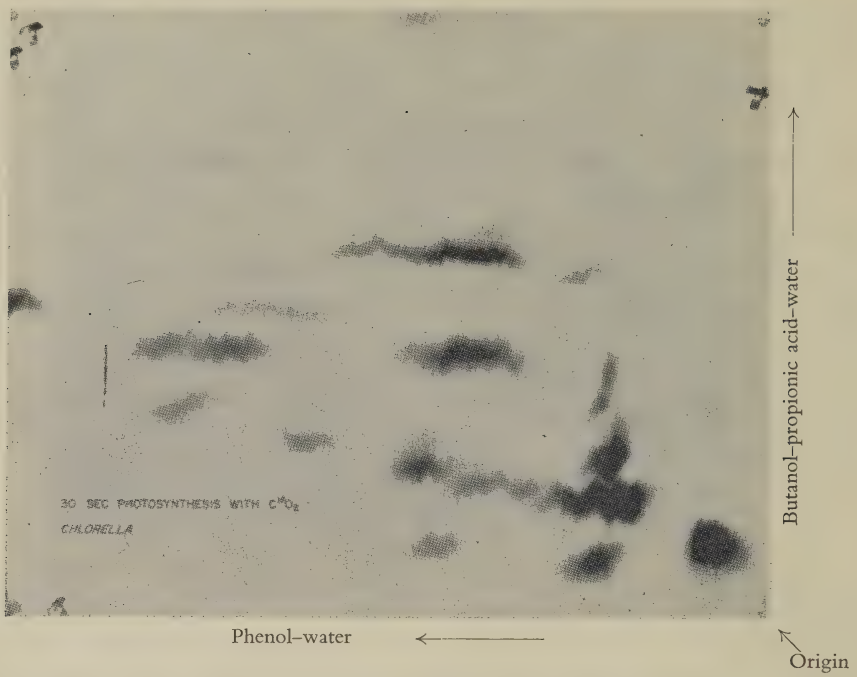

Fig. 4. Chromatogram of extract from algae indicating uptake of radiocarbon during 30 seconds of photosynthesis, using *Chlorella*.

The techniques for doing this were many and varied. It was already clear that the coordinates of a particular spot in a particular chromatogram already could be interpreted in terms of chemical structure in a general way, but this was far from sufficient for identification. Our usual procedure was to seek other properties of the material on the paper, such as fluorescence or ultraviolet absorption if there was enough of it. More commonly it was necessary to elute the material from that part of the paper, as defined by the black area of the film, to perform chemical operations on the eluted material and then rechromatograph the product to determine its fate.

From a succession of such operations the chemical nature of the original material could gradually be evolved, and final identification was usually achieved by co-chromatography of the tracer amount of unknown material with carrier, or macroscopic, amounts of the authentic, suspected compound. A suitable chemical test was then performed on the paper to which the authentic material alone would respond since it was the only material present in sufficient amount. If the response produced by the authentic mate-

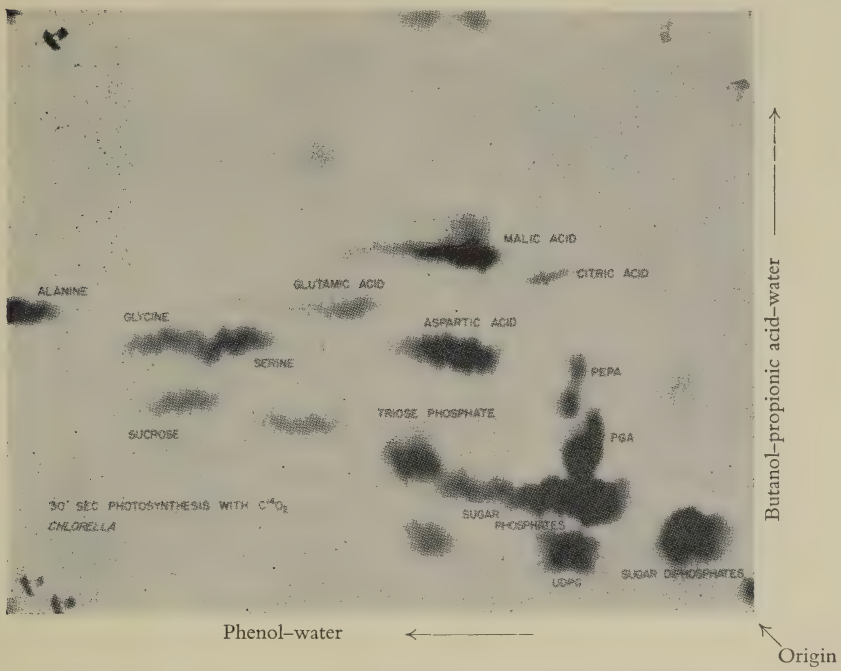

Fig. 5. Chromatogram of extract from *Chlorella* indicating uptake of radioactive carbon during 30 seconds of photosynthesis. UDPG = Uridine diphosphoglucose; PEPA = Phosphoenolpyruvic acid; PGA = Phosphoglyceric acid.

rial coincided exactly with the radioactivity on the paper in all its details, we could be quite confident of the identity of the radioactive compound with the added carrier.

In this way after some ten years of work by many students and collaborators, beginning with Dr. Andrew A. Benson, we were able to place names on a large number of black spots on Fig. 4, as shown in Fig. 5. It is perhaps worth noting that these two chromatograms are duplicate chromatograms of the same extract and are not identical chromatograms, and the degree of reproducibility of the procedure is thus established[8].

Development of the Carbon Reduction Cycle

Phosphoglyceric acid as the first product

It was thus already clear that in only thirty seconds the carbon has passed into a wide variety of compounds and that we would have to shorten the

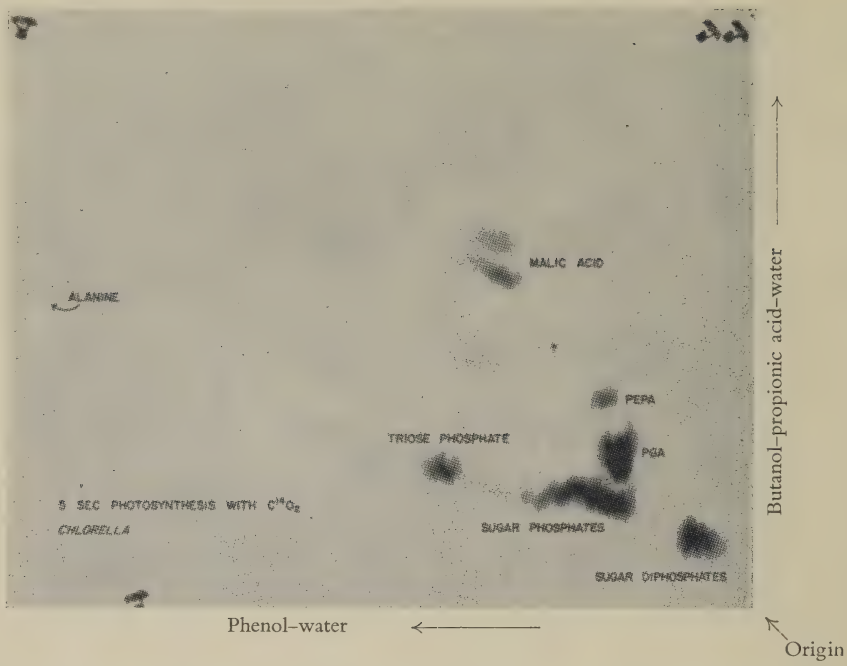

Fig. 6. Chromatogram of extract from *Chlorella* indicating uptake of radiocarbon during 5 seconds of photosynthesis.

exposure time in order to get some clue as to the earliest compounds into which CO_2 is incorporated. This we did in a systematic way, and the result of a five-second exposure is shown in Fig. 6. Here we began to see the dominance of the sugar- and sugar-acid phosphates. In shortening the exposure time still further, it became quite clearly apparent that a single compound dominated the picture in fractions of a second, amounting to over eighty or ninety per cent of the total fixed radioactive carbon. This compound was phosphoglyceric acid. That the phosphoglyceric acid was not the result of the killing procedure but was actually present in the living organism is demonstrated by the fact that when CO_2 is fixed by pre-illuminated algae in the dark under conditions in which not much of the phosphoglyceric acid can be reduced to the sugar level, a good fraction of the three-carbon fragment appears as alanine, as is shown in Fig. 7. Alanine is a stable compound and is not likely to be formed from precursors by merely dropping the algae into the alcohol, and we can be confident that it was present in the living algae. In addition, a wide variety of killing procedures gave the same result.

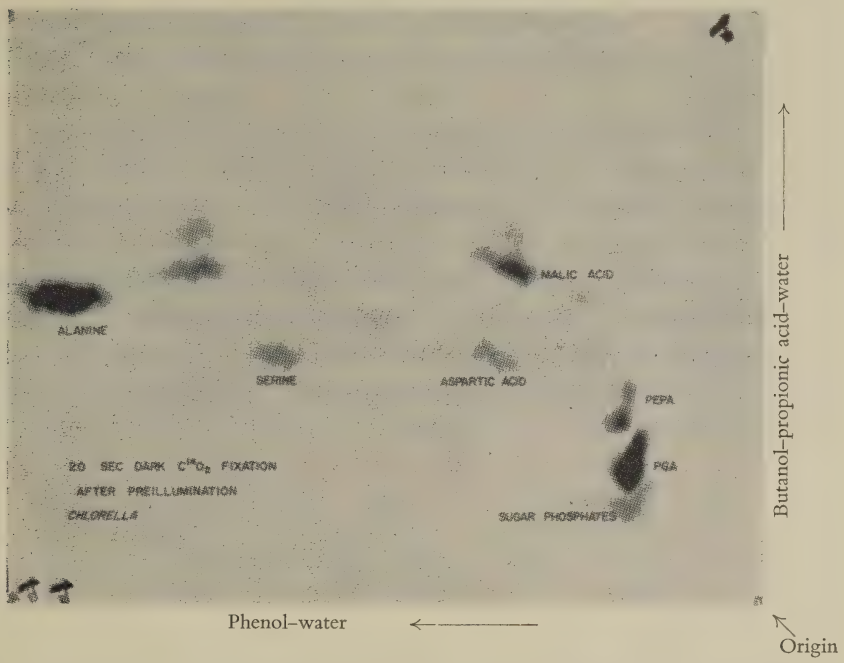

Fig. 7. Chromatogram of extract from *Chlorella* indicating uptake of radiocarbon, 20 seconds dark fixation after pre-illumination.

Thus the presence of phosphoglyceric acid itself in the living plant can be confidently presumed.

Labeling of hexose

Among the earliest sugar phosphates which are shown in Fig. 6 are the triose phosphate and the hexose phosphate, and thus the succession from phosphoglyceric acid to hexose was immediately suggested. Now, the phosphoglyceric acid being a three-carbon compound (and the hexose with its six carbon atoms) required further examination to determine which of the three-carbon atoms were radioactive and in what order they became so. The same information was, of course, required for the hexose as well.

The PGA (phosphoglyceric acid) is readily taken apart following the hydrolysis of the phosphate group, usually with acid, by oxidation, first with periodic acid under conditions which will produce the beta-carbon as formaldehyde and which can be separated as the dimedon compound. The residual two-carbon fragment may be further oxidized with the same reagent,

or better with lead tetra-acetate, to produce CO_2 from the carboxyl group and formic acid from the alpha-carbon atom. These are separately collected and counted.

We were thus able to show that in the very shortest time most of the radioactivity appears in the carboxyl group of the PGA and that radioactivity appears in the alpha- and beta-carbon atoms very nearly equally at later times.

A degradation of the hexose sugar showed that the earliest carbon atoms to be labeled were 3 and 4 (and these approximately equally, although not necessarily exactly so) followed by labeling in carbon atoms 1 and 2, and 5 and 6. The obvious relationship, then, between phosphoglyceric acid and the hexose was the one shown in Fig. 8: after reduction of PGA to "phospho-

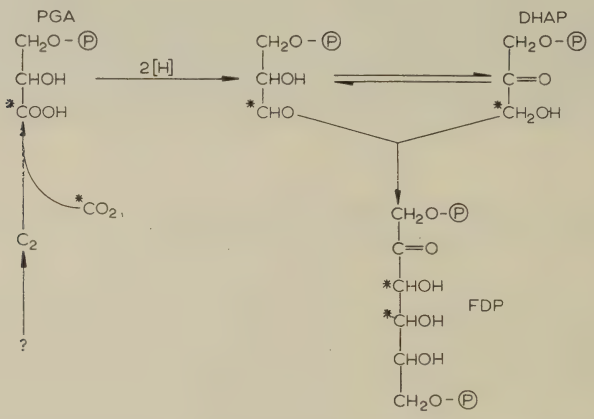

Fig. 8. Path of carbon from carbon dioxide to hexose during photosynthesis.

glyceraldehyde and its conversion to the ketone, the two are condensed by an aldolase reaction to give fructose diphosphate. This places the labeling of the hexose in the center of the molecule.

It is interesting to note that rather early in the fixation sequence a compound appeared which moved extremely slowly in both solvents (that is, remained near the origin particularly in the acid solvent) and which upon extremely mild hydrolysis produced only labeled glucose. This ease of hydrolysis was even greater than that of glucose-1-phosphate, but the material was not glucose-1-phosphate. It was much later shown to be the nucleoside diphosphoglucose, uridine diphosphoglucose, (UDPG in Fig. 5) and its part in the synthesis of sucrose itself deduced from the presence of traces of sucrose

phosphate, later searched for and found. The relationship, then, between phosphoglyceric acid and sucrose is illustrated in Fig. 9.

Origin of phosphoglyceric acid

We now are ready to return to the question of the origin of the PGA itself. Here we were led, by what appeared to be an obvious kind of arithmetic, to seek a compound made of two carbon atoms as a possible acceptor for the radioactive CO_2 to produce the carboxyl-labeled three-carbon compound, phosphoglyceric acid. This search was a vigorous one and extended over a number of years. (Again, a considerable number of students and laboratory

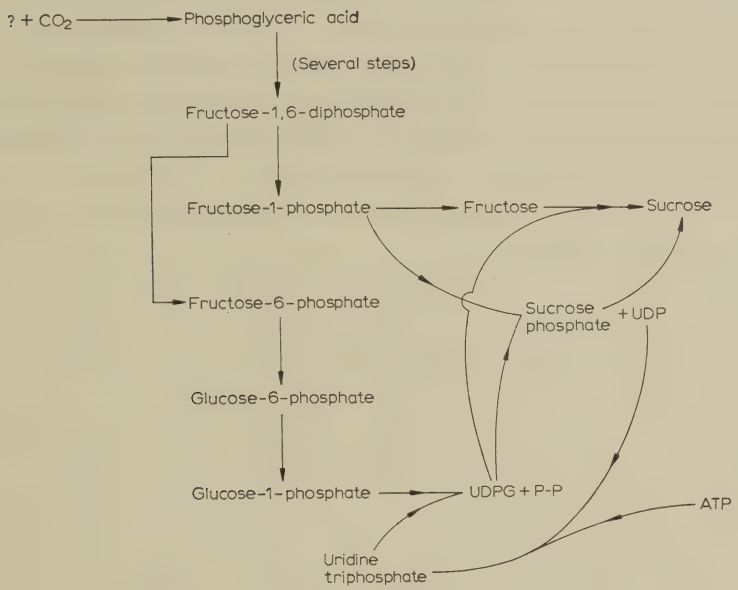

Fig. 9. Relationship between fructose phosphate and sucrose.

visitors were involved.) While free glycolic acid was found under certain very special conditions, these did not correspond to what would be required of the so-called carbon dioxide acceptor. A good many other compounds were identified in the course of this search; particularly among them were a five-carbon sugar, ribulose as its mono- and diphosphate, and a seven-carbon sugar, sedoheptulose as its mono- and diphosphate.

While the relationship of the trioses and hexoses to PGA in a time sequence seemed clear, the sequential relationship of the five-carbon and seven-carbon sugars was not readily determined. In fact, attempts to establish this relationship by ordinary kinetic appearance curves of these two sugars resulted in conclusions on the order of appearance of the pentose, hexose, and heptose which varied from day to day, experiment to experiment, and person to person.

Radioactivity determination of pentose and heptose

The distribution of radioactivity in the pentose and heptose was next determined and that distribution is indicated in Fig. 10 by the number of asterisks on each atom. Thus the No. 3 carbon atom of the ribulose is the first to be labeled, followed by carbon atoms 1 and 2 and finally carbon atoms 4 and 5. In the sedoheptulose, the center three carbon atoms (Nos. 3, 4, 5) were the first to be labeled, followed by carbon atoms 1 and 2, and 6 and 7. Extremely short experiments, of the order of fractions of a second, did show a low value for carbon atom No. 4 in sedoheptulose.

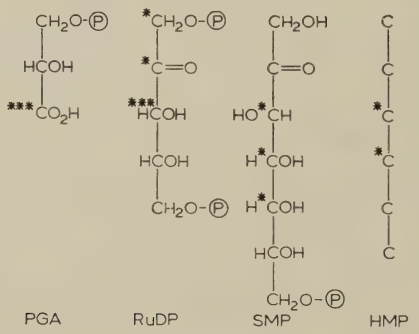

Fig. 10. Distribution of radioactive carbon in certain sugars.

The peculiar labeling in sedoheptulose and the absence of any single one carbon atom as dominating over the others, clearly indicates that it is not formed by a $C_6 + C_1$ addition, that is, not directly from the hexose by the addition of a single carbon atom. The only other alternatives for the formation of the sedoheptulose are the combinations of $C_5 + C_2$ or $C_4 + C_3$. Again, we call on the apparent nearly equal distribution of the center three

carbon atoms of sedoheptulose to indicate that there is no intact element of the five-carbon ribulose present in the sedoheptulose since there is no intact group of five carbon atoms which has the same labeling pattern as we see in the ribulose.

We are therefore forced to seek a $C_4 + C_3$ method for construction of sedoheptulose. The availability of the C_3 fragments is clear enough for the phosphoglyceric acid. The hexose seems, therefore, a possible source of the C_4 fragment which, when combined with a C_3 fragment directly related to PGA, will give rise to a C_7 sugar, sedoheptulose, labeled in the way shown.

This can be done by taking a four-carbon sugar made of carbon atoms No. 3, 4, 5, and 6 (that is, the lower four carbon atoms) of fructose, in which the first two (No. 3 and 4), then, would have the label, and condensing it in an aldol type condensation with the phosphodihydroxyacetone as shown in Fig. 11. If the pool sizes of the tetrose and triose are very small it should be possible to arrive very quickly at a heptose in which the center three carbon atoms are very nearly equally labeled, although one might expect some differences in the shortest times, as we have seen.

The top carbon atoms of the hexose, Nos. 1 and 2, would be combined

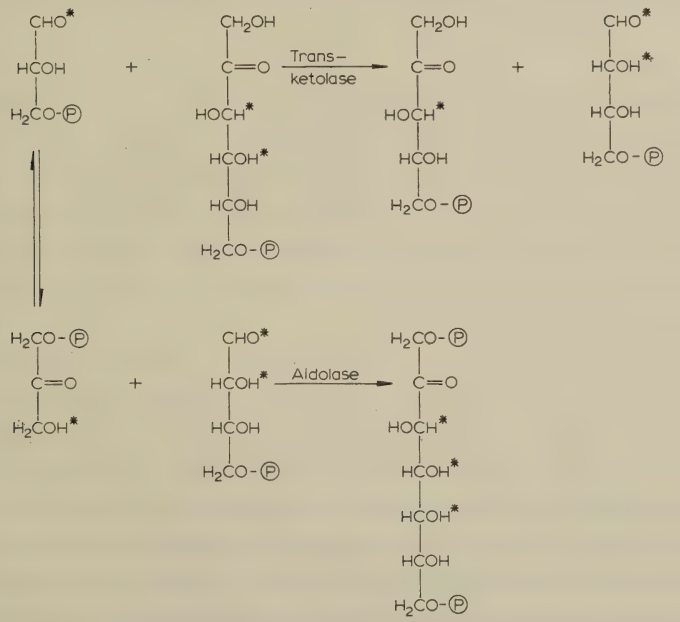

Fig. 11. Formation of a heptose from triose and hexose.

with another triose in the same reaction to produce number 3-labeled pentose.

Identification of the two-carbon acceptor

Accepting this as the source of the heptose, we are now left with the problem of the source of the pentose with its peculiar and unsymmetric labeling pattern. Again, we call on our simple arithmetic, and discover that the pentose can be made by losing a carbon atom from the hexose, or by building it up from smaller fragments, i.e., by adding a C_1 to a C_4 fragment, or by adding a C_2 to a C_3 fragment. Here, again, we can call upon the lack of relationship between the pentose labeling and any five-carbon sequence in the hexose to eliminate from further consideration the construction of the pentose by loss of a terminal carbon from the hexose. Furthermore, of course, this would be a step backwards in our construction program.

We are left, then, with the only remaining alternative for the construction of the pentose, namely, the combination of a C_3 with a C_2 fragment. Again, the source of C_3 fragments is clear enough, but the question of the source of the C_2 fragment to go with it requires some discussion.

At this point it should be remembered that we have already made a C_5 fragment labeled in the No. 3 carbon atom. The ribulose labeling scheme,

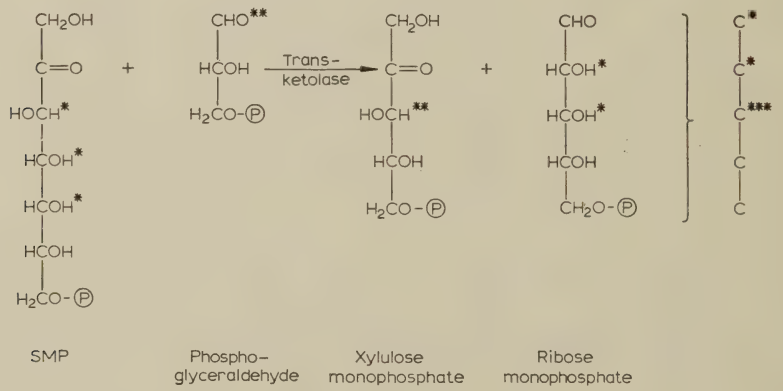

Fig. 12. Proposed scheme for labeling of pentose.

as shown in Fig. 10, indicates that the next label to appear was in carbon atoms No. 1 and 2. Thus it was not until the realization occurred to us that the ribulose which we were degrading and which we obtained from the ribulose diphosphate actually had its origin in two different reactions that it became possible for us to devise a scheme for its genesis.

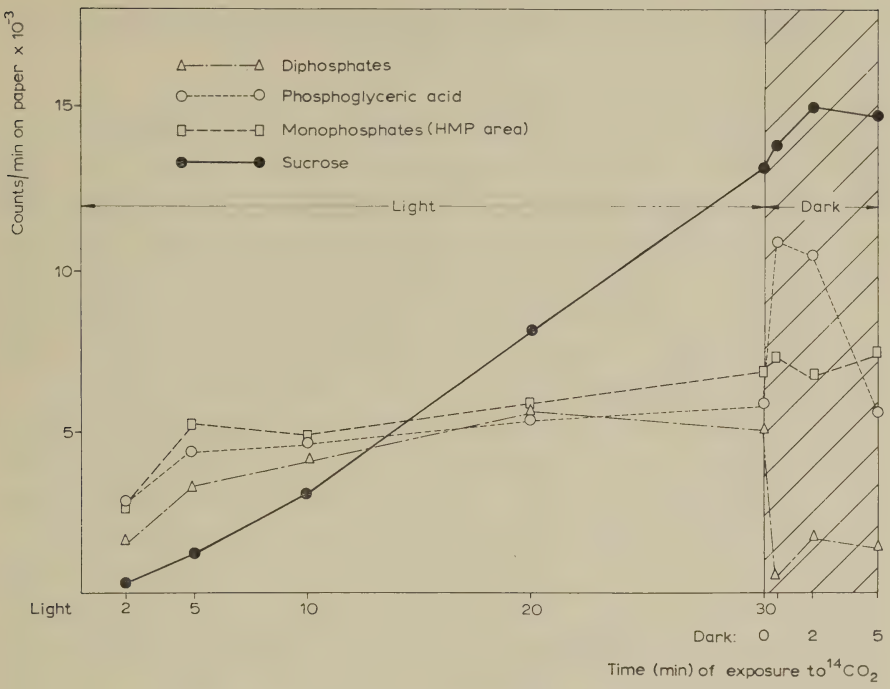

Fig. 13. Effect of light and dark on activities of phosphates and sucrose.

By taking the two-carbon fragment off the top of the sedoheptulose we could make two five-carbon compounds which, taken together with the five-carbon compound already formed, would produce the labeling scheme finally observed in ribulose diphosphate. This is shown in Fig. 12. The enzyme which performs this two-carbon transfer is transketolase and is the same one we have already used to generate the tetrose required for heptose synthesis.

We have thus devised ways of generating from phosphoglyceric acid all of the sugars which appear on our early chromatograms: the triose, the variose pentoses, the various hexoses, and the heptose. The earlier failure to succeed in selecting a specific sequence amongst these compounds is now understandable since all of them, that is, pentose, hexose, and heptose, appear simultaneously following triose. As yet we had not discovered the compound originally presumed to be the C_2 compound, to which the carbon dioxide may be added in order to produce carboxyl-labeled phosphoglyceric acid.

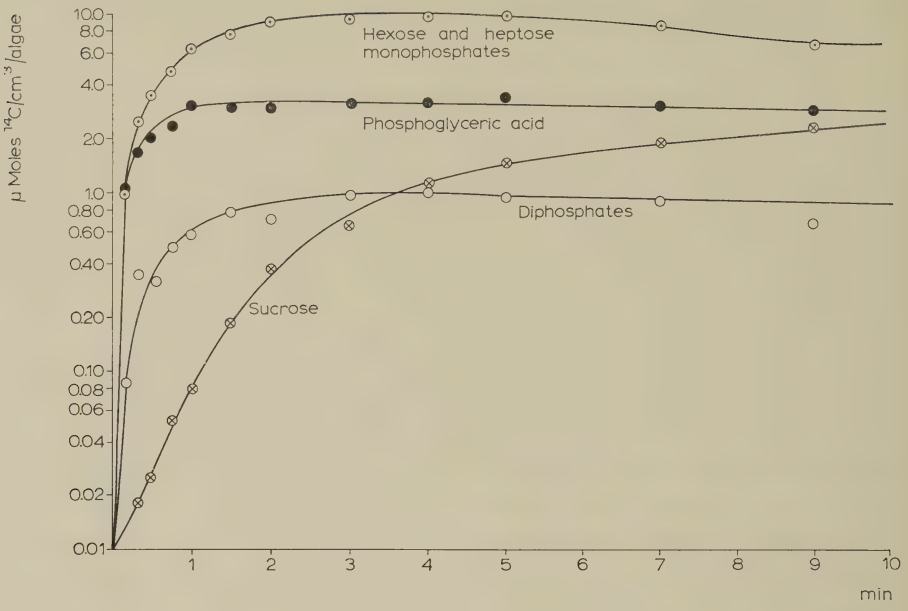

Fig. 14. Appearance of carbon-14 in phosphoglyceric acid and sugar phosphates *vs.* time of photosynthesis with $^{14}CO_2$.

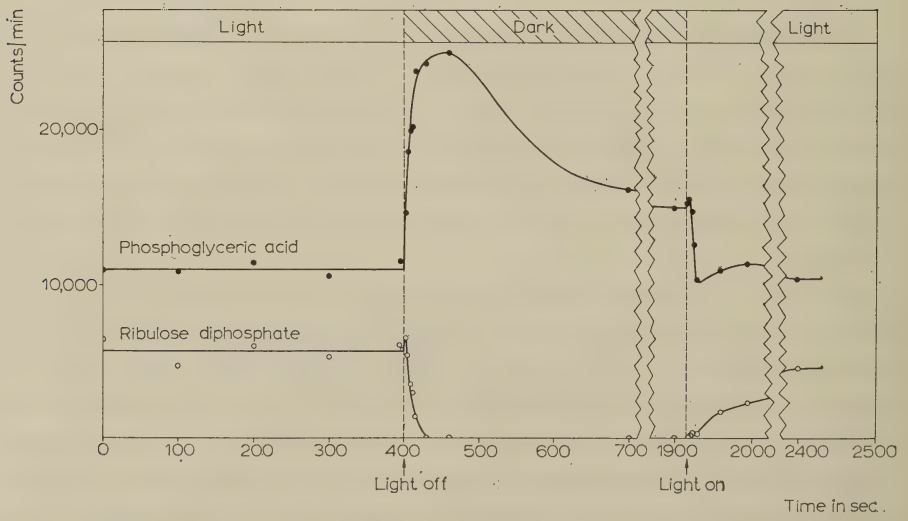

Fig. 15. Light–dark changes in concentrations of phosphoglyceric acid and ribulose diphosphate.

Carbon-fourteen Saturation Experiments: Changes in Steady State and Transients

In order to do this we devised a different type of experiment. We recognized quite early that most of the compounds which we have so far mentioned, aside from sucrose, saturate with radioactivity very quickly, and yet the amount of these materials present in the plant at any one time is small and does not change. This suggested to us a method for discovering not only how the light might operate on the PGA, but also how the PGA might arise. We could use the radioactivity saturation levels for these compounds through which carbon was flowing to measure the total amount of active pool size of these compounds in the plant. We could then change one or another external variable and follow the resultant changes in the pool size of these compounds through which we knew the carbon to be flowing.

The first, most obvious and easiest external variable to be changed was the light itself. Fig. 13 shows the first set of data taken by Peter Massini which not only demonstrates the early saturation but also the effect of the light on the pool transients[9]. Here you can see that the PGA and the sugar phosphate are indeed very quickly saturated but that the sucrose is not. It is apparent that upon turning off the light there is an immediate and sudden rise in the level of PGA accompanied by a corresponding fall in the level of the diphosphate area which was primarily ribulose diphosphate.

Here we had our first definitive clue as to the origin of the phosphoglyceric acid. It would appear that it came as a result of a dark reaction between the ribulose diphosphate and carbon dioxide. Figs. 14 and 15, based upon the work of Dr. James A. Bassham[10,11], show what can be done with this technique when we know what we are doing. Here you can see the large number of points obtainable and the very much smoother curves with their clear saturation points well defined. On Fig. 15 we see again very sharply shown the transient rise in the PGA pool size and the disappearance of ribulose diphosphate when the light is turned off, clearly defining the relationship between these two compounds.

We can now formulate the cyclic system driven by high-energy compounds produced in the light, acting upon phosphoglyceric acid which, in turn, is made as a result of a reaction between ribulose diphosphate and carbon dioxide as shown in Fig. 16. The triose phosphate then undergoes a series of condensations and sugar rearrangements, represented by the letters A and B and including the pentose and heptose rearrangement which we

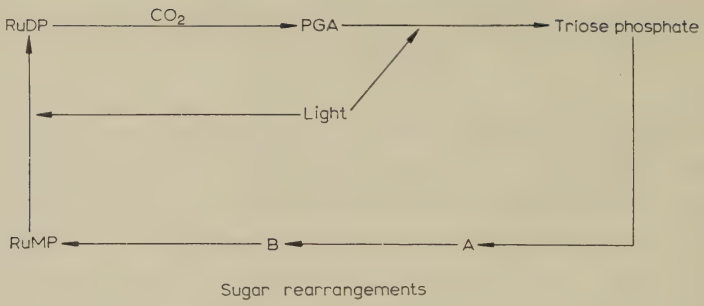

Fig. 16. Formation of five-carbon sugars from ribulose diphosphate.

have just discussed, leading back again to a ribulose monophosphate which is then phosphorylated to ribulose diphosphate, thus completing the cycle.

By turning off the light we have, in effect, blocked the easy conversion of phosphoglyceric acid to triose without any reduction in the rate of formation of PGA from ribulose diphosphate. This should result in an immediate rise in the amount of PGA and an immediate fall in the amount of ribulose since it no longer can be produced in the dark.

Such a scheme allows us to predict another transient, namely, the one which would result if, in the presence of light, that is, in the presence of the high-energy compounds required to drive the cycle, we suddenly diminished the availability of carbon dioxide, putting a block between ribulose and the phosphoglyceric acid. The prediction, which resulted here in the first transients, would be the accumulation of ribulose and the disappearance of PGA. These two transients would then make their way back through the cycle, the fall in PGA in a clockwise direction, the rise in ribulose in a counterclockwise direction, thus making for oscillating transients. The result of such an experiment performed by Dr. Alexander T. Wilson[12] is shown in Fig. 17. Here it is quite clear that the sudden reduction in the CO_2 results in a drop in the size of the PGA pool associated with a rise in the ribulose. It is interesting to note that the triose is the last to rise and the first to fall, as predicted from its position in the cycle.

The Photosynthetic Carbon Cycle

We can now arrange all of the individual steps we have separately discussed in a sequence to produce the photosynthetic carbon cycle as shown in Fig.

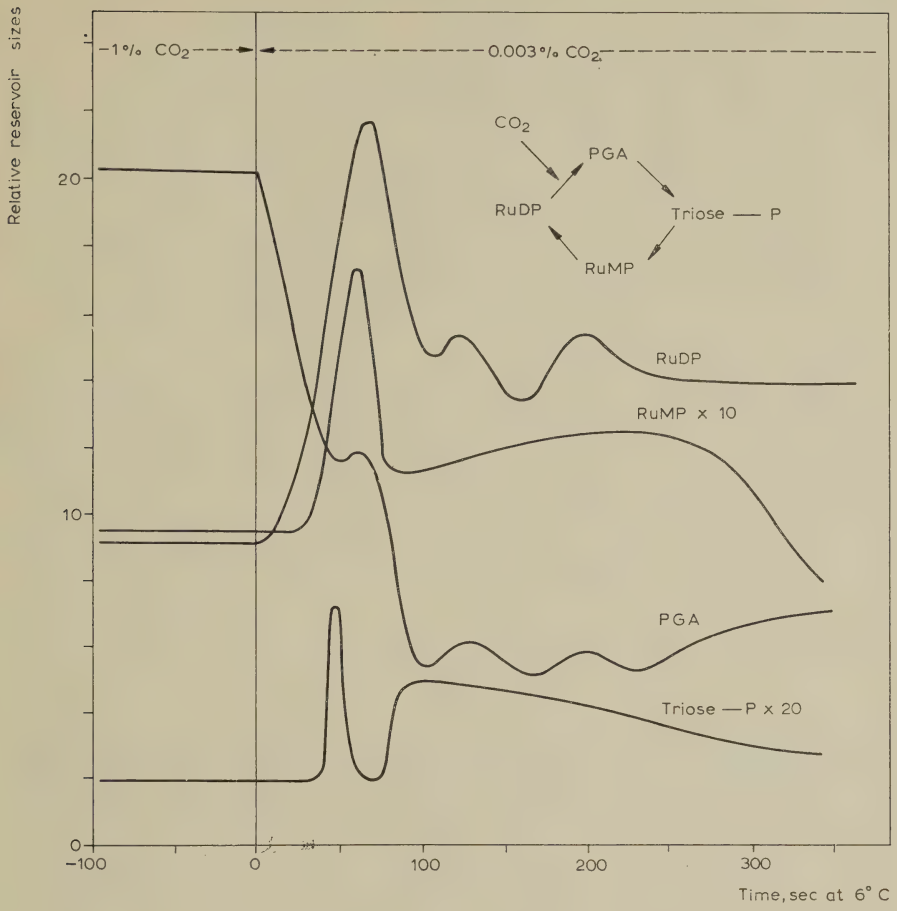

Fig. 17. Transients in the regenerative cycle.

18. One compound is shown therein as an intermediate lying between ribulose diphosphate and phosphoglyceric acid. This is a branched chain alpha-hydroxy-beta-keto sugar acid. As yet this compound has not been isolated as a separate entity. The enzyme system catalyzing the reaction of ribulose diphosphate to PGA has been isolated and purified, but as yet we have been unable to break its reaction down into two steps[13]. If this intermediate is present, it is present in extremely small amounts as the free compound, if any at all, and its hydrolysis to produce two molecules of phosphoglyceric acid takes place extremely rapidly in the isolated enzyme system and even in the living plant itself.

The mechanism for the carboxydismutase (the enzyme responsible for the

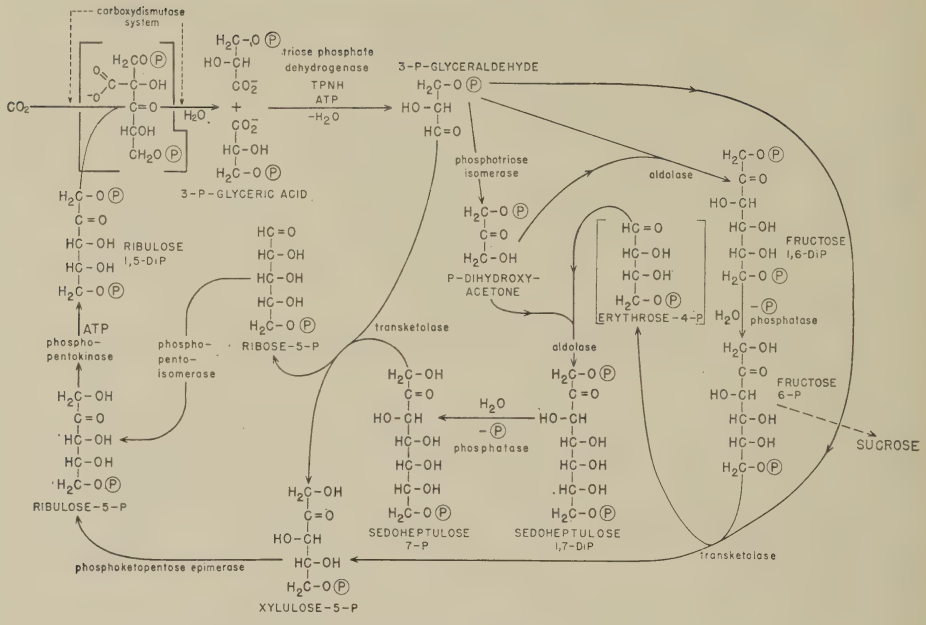

Fig. 18. The photosynthetic carbon cycle.

formation of PGA from ribulose diphosphate) reaction is formulated in Fig. 19. Here the intermediate is split by hydrolysis to two molecules of phosphoglyceric acid. However, in our earlier work the possibility of a reductive fission at the same point to give one molecule of triose and one molecule of phosphoglyceric acid was considered. It was rejected in favor of the hydrolytic splitting because of our failure to find any evidence of the intermediate. However, more recent, very careful kinetic analysis of the carbon flow rates by Dr. James A. Bassham[11,14] has suggested that the reductive split may indeed participate in the reaction to some extent while the light is actually on. This path is indicated in Fig. 20 by the dotted line from the presumed C_6 keto acid.

Quantum Conversion in Photosynthesis

As you can see from the various levels of the schematic drawing of the photosynthetic carbon cycle (Figs. 18 and 20), the energy required to drive the synthetic sequence from carbon dioxide to carbohydrate and the many other

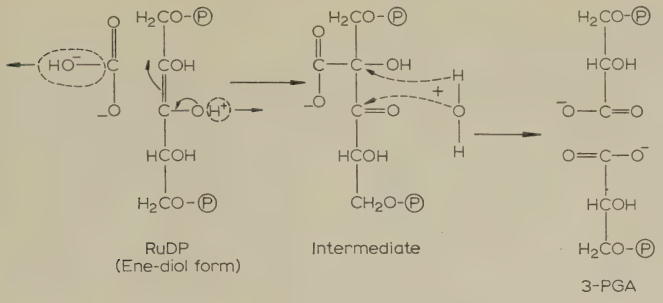

Fig. 19. Mechanism of the carboxydismutase reaction.

reduced carbon materials which can be derived from the cycle is delivered to it in the form of a number of compounds of relatively high chemical potential in the aerobic aqueous system in which the plant operates. The particular ones with which we can actually drive the photosynthetic carbon cycle in the absence of light but in the presence of all the initial enzymes and substrates[15] are triphosphopyridine nucleotide (TPNH) and adenosine triphosphate (ATP) whose structures are shown in Fig. 21. The primary quantum conversion of the light absorbed by chlorophyll will result in materials which can ultimately give rise to such substances as these.

That light energy might be readily converted into chemical potential as ATP independent of CO_2 reduction and its re-oxidation was clearly in-

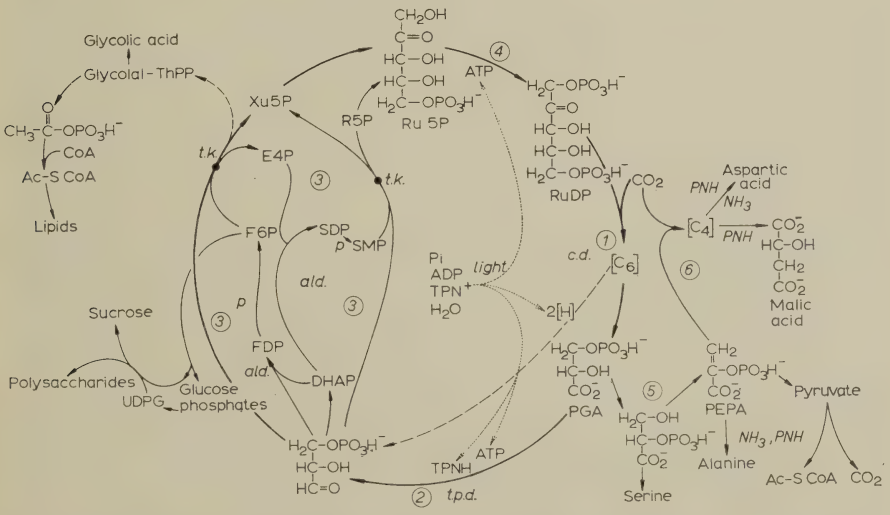

Fig. 20. The photosynthetic carbon cycle and its relation to quantum conversion and to succeeding biosynthesis.

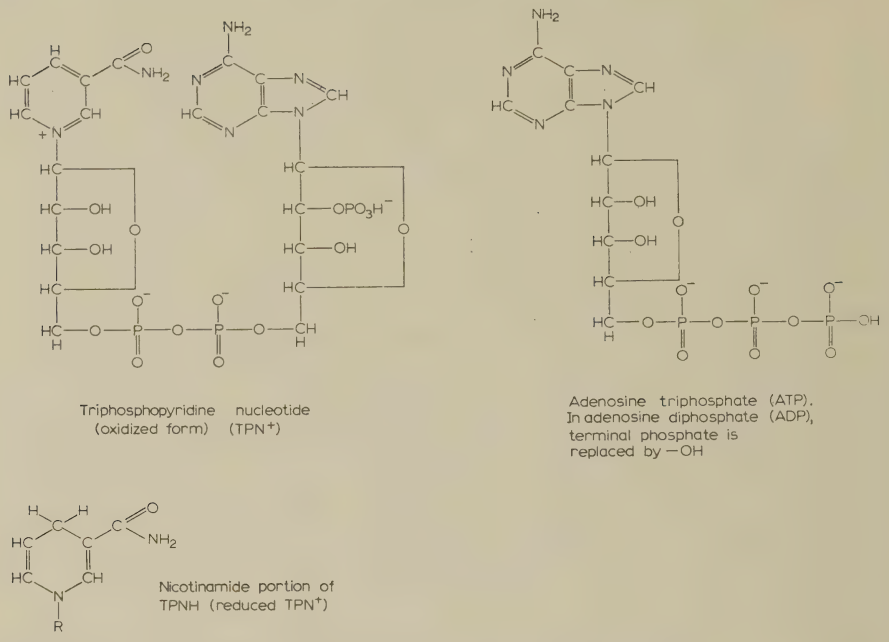

Fig. 21. Structure of TPNH and ATP, the two compounds which are used to drive the photosynthetic carbon cycle in the absence of light and the presence of enzymes.

dicated in whole-cell experiments of Goodman and Bradley[16] and first demonstrated as independent of oxygen by Frenkel[17] with bacterial chromatophores. A corresponding anaerobic demonstration of ATP production by green plant chloroplasts was made by Arnon[18]. The ability of chloroplasts to photoreduce pyridine nucleotide was demonstrated by Ochoa and Vishniac[19]. But the precise nature of the primary quantum conversion act whose products ultimately give rise in a dark reaction to ATP and TPNH is still a matter of speculation[20,21].

The apparatus which performs the quantum conversion act in the plant, together with all of the carbon reduction enzymes we now know, can be isolated from the intact chloroplasts in the higher plants. The carbon reduction enzymes are very easily washed off the chloroplasts by water, leaving behind only the chlorophyllous quantum conversion equipment. This has a highly ordered structure in which the lamellae are alternating electron-dense and electron-thin materials, as has been shown in many electron micrographs, a few examples of which are shown in Fig. 22 for various organisms.

The next level of structure within the lamellae is only now beginning to be visible to us, and an example is shown[22] for a spinach chloroplast in Fig.

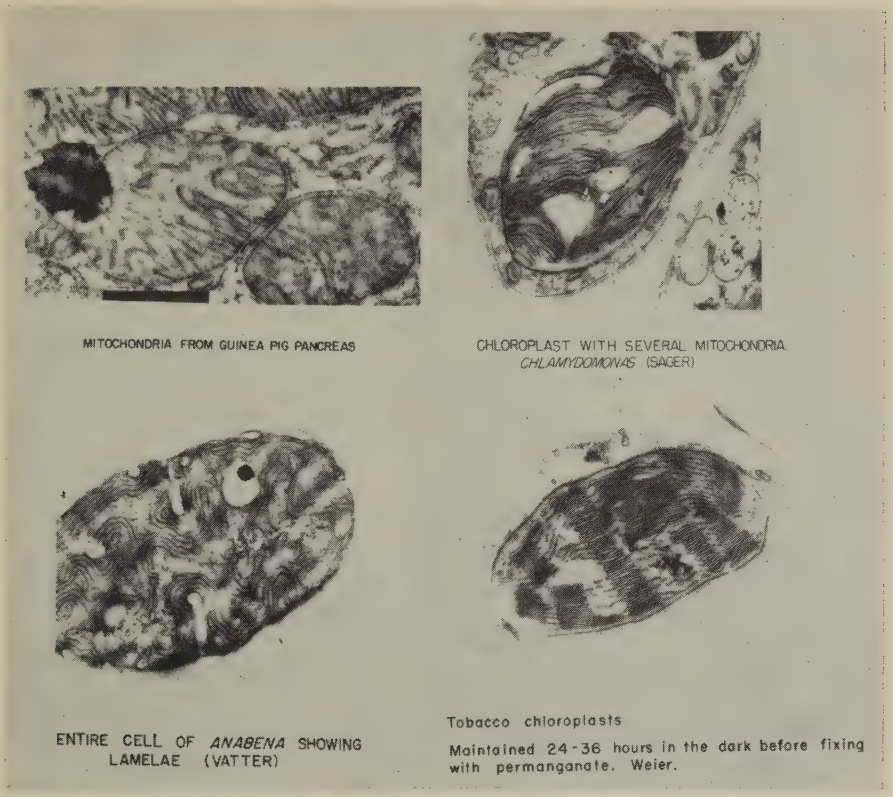
MITOCHONDRIA FROM GUINEA PIG PANCREAS
CHLOROPLAST WITH SEVERAL MITOCHONDRIA
CHLAMYDOMONAS (SAGER)
ENTIRE CELL OF ANABENA SHOWING LAMELAE (VATTER)
Tobacco chloroplasts
Maintained 24-36 hours in the dark before fixing with permanganate. Weier.

Fig. 22. Electron micrographs of chloroplasts from a unicellular green alga, from a blue-green alga, from tobacco, and mitochondria from guinea pig pancreas.

23. Here we can see the lamellae on its flat side showing a granular structure, made up of fairly uniform oblate spheroids which we have called «Quantasomes»; this work was performed by Drs. Roderic B. Park and Ning G. Pon. Within these Quantasomes the chlorophyll itself is highly organized, as we have been able to demonstrate, particularly by electric dichroism experiments performed by Dr. Kenneth H. Sauer[23].

We are now in the midst of trying to determine precisely what happens after the chlorophyll has absorbed the quantum and has become an excited chlorophyll molecule, a problem that involves the physicist and physical chemist, as well as the organic and biochemists. The determination of the next stage in the energy-conversion process is one of our immediate concerns. Either it is an electron transfer process[20], and thus comes close in its

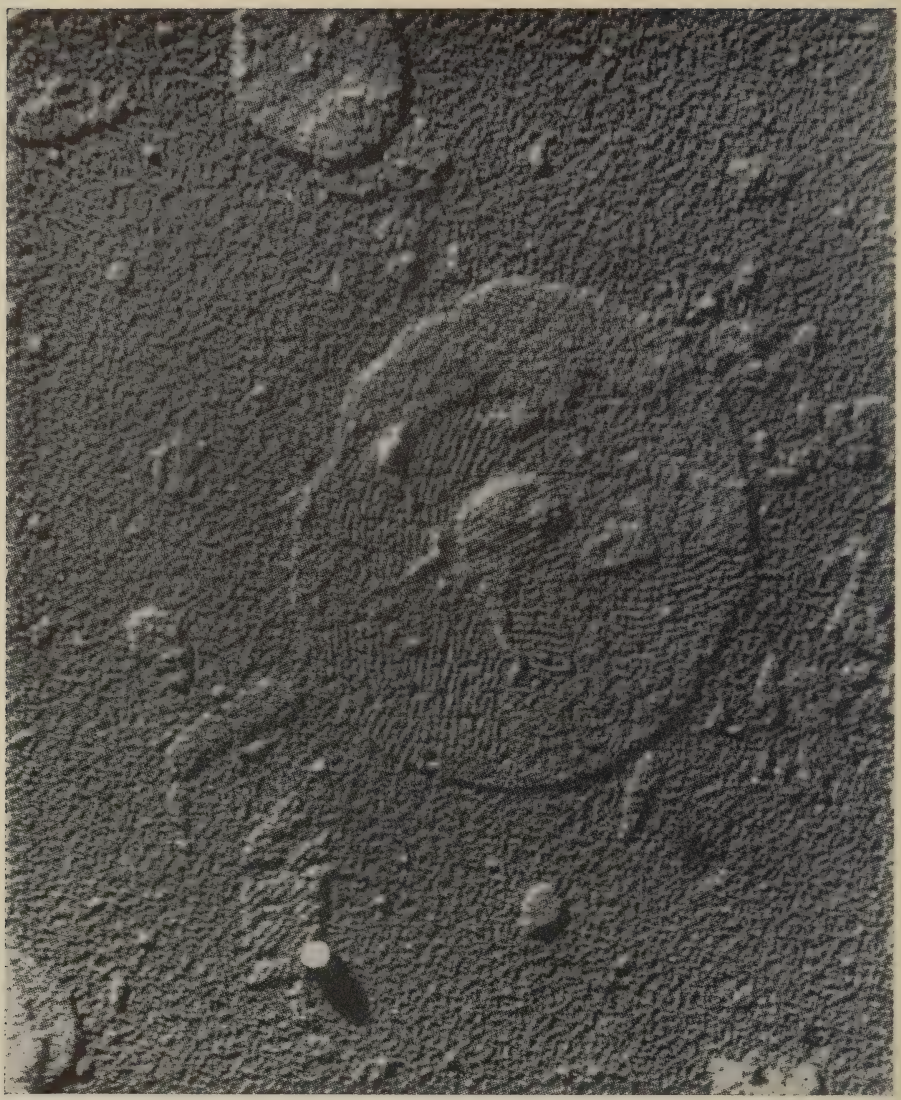

Fig. 23. Quantasomes from spinach chloroplasts. The white spot at lower left is a polystyrene latex marker 880 Å in diameter. The ordered array of quantasomes within a single granum is clearly evident.

further stages to the electron transfer processes which are being explored in mitochondria[24] or it is some independent non-redox method of energy conversion[21]. This remains for the future to decide.

Chemical biodynamics, involving as it does, the fusion of many scientific

disciplines, will play a role in this problem, as it has in the elucidation of the carbon cycle. It can be expected to take an increasingly important place in the understanding of the dynamics of living organisms on a molecular level.

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Biography

Melvin Calvin was born in St. Paul, Minnesota, April 8, 1911, of Russian emigrant parents. He received the B.S. degree in Chemistry in 1931 at the Michigan College of Mining and Technology, and the Ph.D. degree in Chemistry from the University of Minnesota in 1935. He spent the academic years 1935–1937 at the University of Manchester, England. He began his academic career at the University of California at Berkeley in 1937, as an instructor, and has been a full professor since 1947. He has served as Director of the bio-organic chemistry group in the Lawrence Radiation Laboratory since 1946. This group became the Laboratory of Chemical Biodynamics in 1960.

He has been the recipient of a number of medals, awards, and lectureships, and holds membership in numerous learned societies. In addition, he has been elected to the National Academy of Sciences, the American Philosophical Society, the American Academy of Arts and Sciences, the Royal Society of London, the Royal Netherlands Academy of Sciences and Letters, and the German Academy of Scientists, Leopoldina. He holds honorary D.Sc. degrees from Michigan College of Mining and Technology, the University of Nottingham, Oxford University, and Northwestern University.

His scientific life began with a thesis on the electron affinity of halogens, done under the direction of Professor George A. Glocker at the University of Minnesota and completed in 1935. The following two-year postdoctoral period was spent with Professor Michael Polanyi at the University of Manchester, at which time his interest in coordination catalysis, particularly metalloporphyrins, was awakened. This interest is still paramount and has resulted both in theoretical (*The Chemistry of Metal Chelate Compounds*) and practical (oxygen-carrying synthetic chelate compounds) applications. The investigation of the electronic, photoelectric and photochemical behaviour of such materials now occupies a good fraction of his time.

Upon coming to Berkeley, California, at the invitation of Professor Gilbert N. Lewis, his interest turned to general theoretical aspects of organic molecular structure and behaviour. There were two prime publications of this

period. The first, with Professor Gilbert N. Lewis, was on *The Color of Organic Substances*, and the second, with Professor G. E. K. Branch, was *The Theory of Organic Chemistry*. It was from these men that the fundamental interest in the behaviour of organic molecules in their most detailed terms was derived.

This interest combined with the earlier one on the catalytic behaviour of coordination compounds were the natural parents of his present preoccupation with the problem of photosynthesis. The ready availability of carbon-14 which began in 1945 channeled the early work to development of techniques for its use (*Isotopic Carbon*) and its application to the exploration of photosynthetic carbon dioxide reduction (*The Path of Carbon in Photosynthesis*).

An extension of his interest from here into the general problems of biology was unavoidable, and thus his laboratory is at present peopled by emigrants from all areas of science on both sides of chemistry – physics on the one hand and biology on the other.

Dr. Calvin is married to the former Genevieve Jemtegaard, daughter of Norwegian emigrant parents, they have two daughters, Elin and Karole, and one son, Noel.

Chemistry 1962

MAX FERDINAND PERUTZ

JOHN COWDERY KENDREW

«for their studies of the structures of globular proteins»

Chemistry 1962

Presentation Speech by Professor G. Hägg, member of the Nobel Commitete for Chemistry of the Royal Swedish Academy of Sciences

Your Majesties, Your Royal Highnesses, Ladies and Gentlemen.

In the year 1869 the Swedish chemist Christian Wilhelm Blomstrand wrote, in his at that time remarkable book *Die Chemie der Jetztzeit* (Chemistry of Today):

« It is the important task of the chemist to reproduce faithfully in his own way the elaborate constructions which we call chemical compounds, in the erection of which the atoms serve as building stones, and to determine the number and relative positions of the points of attack at which any atom attaches itself to any other; in short, to determine the distribution of the atoms in space. »

In other words, Blomstrand gives here as his goal the knowledge of how compounds are built up from atoms, i.e. knowledge of what is nowadays often called their « structure ». Moreover, structure determination has been one of the biggest tasks of chemical research, and has been approached using many different techniques. For several reasons, the structure determination of carbon compounds, the so-called organic compounds, experienced an initial rapid development. At this stage the techniques were generally those of pure chemistry. One drew conclusions from the reactions of a compound, one studied its degradation products, and tried to synthesize it by combining simpler compounds. The structure thus arrived at, however, was in general rather schematic in character; it showed which atoms were bonded to a given atom, but gave no precise values for interatomic distances or interbond angles. However, for an up-to-date treatment of the chemical bond and in order to derive a correlation between structure and properties, these values are needed, and they can only be obtained using the techniques of physics.

The physical method which, more than any other, has contributed to our present-day knowledge of these mutual dispositions of the atoms is founded on the phenomenon which occurs when an X-ray beam meets a crystal. This phenomenon, called diffraction, results in the crystal sending out beams of X-rays in certain directions. These beams are described as reflections. The

directions and intensities of such reflections depend on the type and distribution of the atoms within the crystal, and can therefore be used for structure determination. It is 50 years ago this year since Max von Laue discovered the diffraction of X-rays by crystals, a discovery for which he was awarded the 1914 Nobel Prize for Physics. This work opened up a whole new range of possibilities for studying both the nature of X-rays and the structure of compounds in the solid state. The initial application of structure determination was developed first and foremost by the two English scientists, Bragg father and son, and as early as 1915 they were rewarded with the Nobel Prize for Physics. The techniques have since been considerably refined, and it has been possible to solve more and more complicated structures. However, considerable difficulties were encountered as soon as any other than very simple structures were considered. There is no simple general way of progressing from experimental data to the structure of the compound under investigation. Moreover, the mathematical calculations are exceedingly time-consuming. However, by about the middle of the 1940's a point had been reached where it was becoming possible to carry out X-ray determinations of the structures of organic compounds which were so complicated that they defied all attempts using classical chemical methods.

In 1937 Max Perutz performed some experiments in Cambridge to find out whether it might be possible to determine the structure of haemoglobin by X-ray diffraction, since no other method could be imagined for this purpose. Sir Lawrence Bragg, who tirelessly continued the work begun jointly with his father, in 1938 became the head of the Cavendish Laboratory in Cambridge. When he saw the results obtained by Perutz, he encouraged him to continue and has ever since lent a very efficient support. Haemoglobin belongs to the proteins which play such an enormous part in life processes, and which are a basic material in living organisms. Haemoglobin is a component of the red blood corpuscles. It contains iron which can take up oxygen in the lungs and later give it up to the body's other tissues. Haemoglobin is counted among the globular proteins, whose molecules are nearly spherical. It was chosen for the initial attempt, partly because it could develop good crystals, and partly because the haemoglobin molecule is quite small for a protein molecule. About ten years later, John Kendrew joined Perutz' research group, and the task allotted to him was to try to determine the structure of myoglobin. Myoglobin is another globular protein, closely related to haemoglobin, but with a molecule only a quarter as large. It is found in the muscles, and enables oxygen to be stored there. Particularly large

amounts of myoglobin are found in the muscular tissues of whales and seals, which need to be able to store large quantities of oxygen when diving.

However, Perutz and Kendrew encountered considerable difficulties. In spite of exceptionally comprehensive work, the result was not forthcoming until 1953, when Perutz succeeded in incorporating heavy atoms, namely those of mercury, into definite positions in the haemoglobin molecule. By this means the diffraction pattern is altered to some extent, and the changes can be utilized in a more direct structure determination. The method was already known in principle, but Perutz applied it in a new way, and with great skill. Kendrew also succeeded, by an alternative method, in incorporating heavy atoms, generally mercury or gold, into the myoglobin molecule, and could subsequently proceed in an analogous manner.

A necessary condition for this technique is that the addition of the heavy atoms should not alter the positions of the other atoms of the molecule within the crystal. In this connection it is simply because of its enormous dimensions that the molecule remains practically unaltered. Bragg has rather aptly said that « the molecule takes no more notice of such an insignificant attachment than a maharaja's elephant would of the gold star painted on its forehead ».

But even if the path was now open for a direct structure determination of haemoglobin and myoglobin, there was still an enormous amount of data to be processed. Myoglobin, the smaller of the two molecules, contains about 2,600 atoms, and the positions of most of these are now known. But for this purpose, Kendrew had to examine 110 crystals and measure the intensities of about 250,000 X-ray reflections. The calculations would not have been practicable if he had not had access to a very large electronic computer. The haemoglobin molecule is four times as large, and its structure is known less thoroughly. In both cases, however, Kendrew and Perutz are currently collecting and processing an even greater number of reflections in order to obtain a more detailed picture.

As a result of Kendrew's and Perutz' contributions it is thus becoming possible to see the principles behind the construction of globular proteins. The goal has been reached after twenty-five years' labour, and initially with only modest results. We therefore admire the two scientists not only for the ingenuity and skill with which they have carried out their work, but also for their patience and perseverance, which have overcome the difficulties which initially seemed insuperable. We now know that the structure of proteins can be determined, and it is certain that a number of new determina-

tions will soon be carried out, perhaps chiefly following the lines which Perutz and Kendrew have indicated. It is fairly certain that the knowledge which will thus be gained of these substances which are so essential to living organisms will mean a big step forward in the understanding of life processes. It is thus abundantly clear that this year's prize-winners in chemistry have fulfilled the condition which Alfred Nobel laid down in his will; they have conferred the greatest benefit on mankind.

Doctor Kendrew and Doctor Perutz. One of you recently said that today's students of the living organism do indeed stand on the threshold of a new world. You have both contributed very efficiently to the opening of the door to this new world and you have been among the first to obtain a glimpse of it. Through your combined efforts there is now in view, as it has been stated by yourself, a firm basis for an understanding of the enormous complexities of structure, of biogenesis and of the functions of living organisms both in health and disease.

It is with great satisfaction, therefore, that the Royal Swedish Academy of Sciences has decided to award you this year's Nobel Prize for Chemistry for your brilliant achievement.

On behalf of the Academy I wish to extend to you our heartiest congratulations, and now ask you to receive from the hands of His Majesty the King the Nobel Prize for Chemistry for the year 1962.

Max F. Perutz

X-ray analysis of haemoglobin

Nobel Lecture, December 11, 1962

Forty years ago William Lawrence Bragg came here to thank you for awarding the Nobel Prize to himself and to his father, and to tell you how they had brought into being a new and fruitful branch of physics by unravelling the atomic arrangement in crystals of common salt and other simple compounds.[1] Today Sir Lawrence Bragg ranks as one of the fathers of X-ray crystallography, but he has also been something of a scientific father to me personally, and I feel immensely proud that it should now be my own turn, as his former pupil, to thank you for having bestowed on me this supreme honour.

I started my X-ray work on crystalline proteins in the Cavendish Laboratory at Cambridge under J. D. Bernal in 1937, just after he and Dorothy Hodgkin had demonstrated that protein crystals can be made to yield sharp X-ray diffraction patterns which extend to spacings of the order of interatomic distances[2]. Soon afterwards Sir Lawrence Bragg was appointed Cavendish Professor. My X-ray diffraction pictures of haemoglobin at once fired his imagination and enthusiasm. He was fascinated by the idea that the powers of X-ray analysis might be extended to the giant molecules which form the catalysts of living cells. Bragg's determined support made it possible for me, and later for both Kendrew and myself, to carry on with our studies over the many years that were needed to develop X-ray methods suitable for such highly complex structures, and I know that Bragg is overjoyed by the success and the honours that have now rewarded us.

Physical principles

X-ray analysis of proteins is often regarded as a subject comprehensible only to specialists, but the basic ideas underlying our work are simple. A small protein crystal is mounted in a glass capillary to keep it wet (Fig. 1) and is illuminated by a narrow pencil of X-rays. Upon rotating the crystal in certain ways, a regular pattern of diffracted X-rays is produced on a photographic

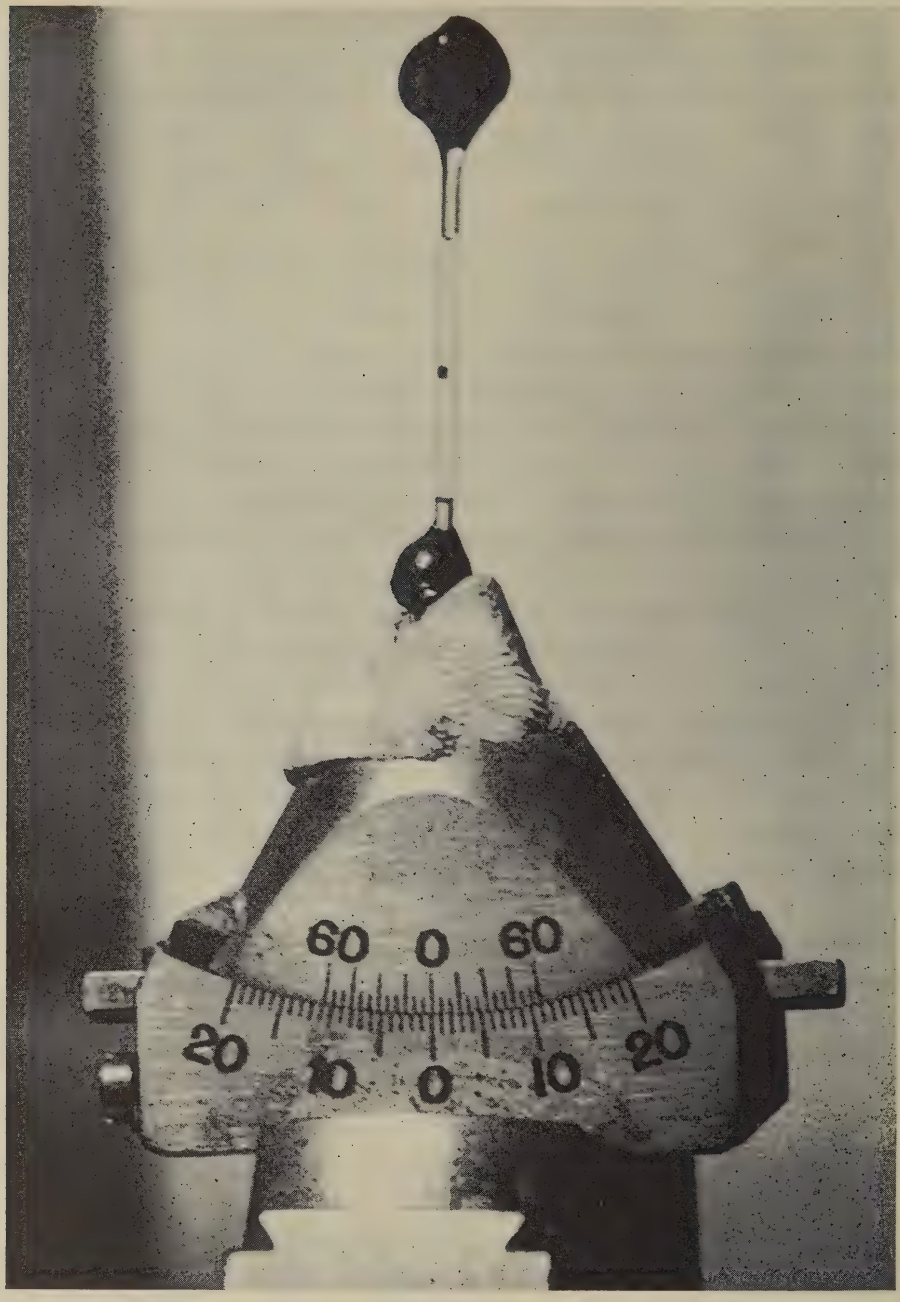

Fig. 1. Haemoglobin crystal in equilibrium with its mother liquor, mounted for X-ray analysis. (The glass capillary is about 1 mm wide.)

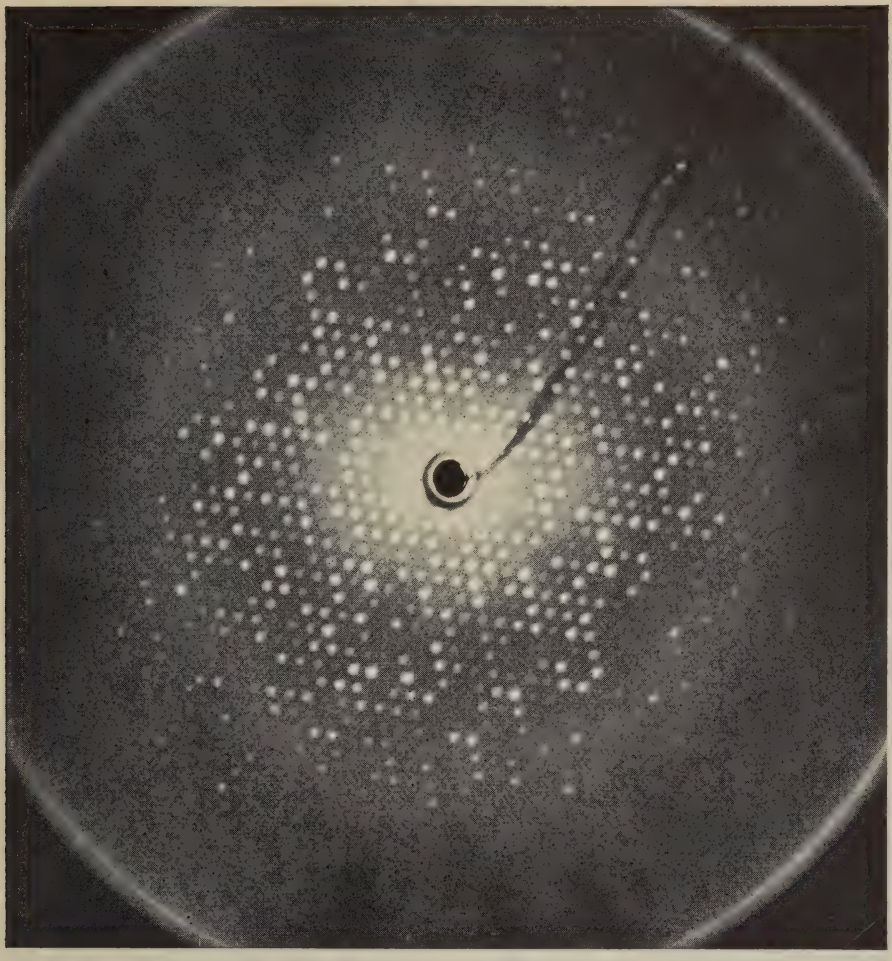

Fig. 2. X-ray diffraction pattern from a haemoglobin crystal, taken with a Buerger Precession Camera. The pattern fades out near the rim of the picture, which corresponds to a spacing of 1.8Å.

film placed behind the crystal (Fig. 2). The spots are seen to lie at the corners of a regular lattice which bears a reciprocal relationship to the arrangement of molecules in the crystal. Moreover, each spot has a characteristic intensity which is determined in part by the atomic arrangement inside the molecule. In his Nobel Lecture (*Les Prix Nobel en 1921–1922*) Sir Lawrence Bragg explained this relationship in the following words:

«It is well known that the form of the lines ruled on a grating has an influence on the relative intensity of the spectra which it yields. Some spectra

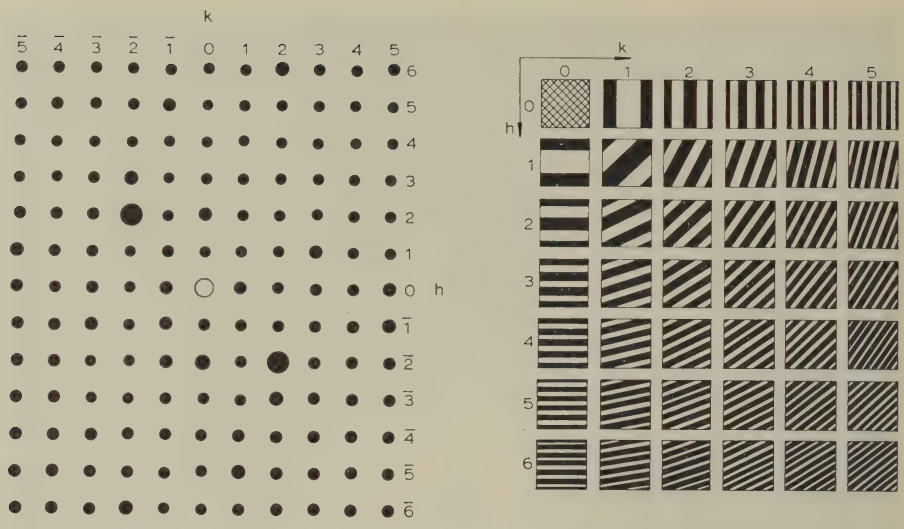

Fig. 3. (*Left*): Array of spots in an X-ray diffraction pattern. The varying intensities are represented by circles of varying size. The orders are given on the margin. (*Right*): Sets of fringes produced by pairs of spots in the diffraction pattern which are symmetrically related about the origin at the centre. The order of the fringes *h* and *k* corresponds to the order of the spots used to produce them. (*Reproduced, by permission, from W. de Beauclair, «Verfahren und Geräte zur mehrdimensionalen Fouriersynthese», Akademie-Verlag, Berlin 1949.*)

may be enhanced, or reduced, in intensity as compared with others. Indeed, gratings are sometimes ruled in such a way that most of the energy is thrown into those spectra which it is most desirable to examine. The form of the line on the grating does not influence the positions of the spectra, which depend on the number of lines to the centimetre, but the individual lines scatter more light in some directions than others, and this enhances the spectra which lie in those directions.

The structure of the group of atoms which composes the unit of the crystal grating influence the strength of the various reflexions in exactly the same way. The rays are diffracted by the electrons grouped around the centre of each atom. In some directions the atoms conspire to give a strong scattered beam, in others their effects almost annul each other by interference. The exact arrangement of the atoms is to be deduced by comparing the strength of the reflexions from different faces and in different orders.»

Thus there should be a way of reversing the process of diffraction, and of getting back from the diffraction pattern to an image of the atomic arrange-

ment. In order to produce such an image, each pair of symmetrically related spots in the X-ray pattern can be made to generate a set of fringes, each fringe having an amplitude proportional to the root of the intensity of the spot (Fig. 3). The fringes should now be superimposed, using either calculation or optical analogues, to give the required image (Fig. 4a).

However, at this stage a complication arises. To obtain the *right* image, each set of fringes must be placed correctly relative to some arbitrarily chosen, common, origin (Fig. 5). At this origin the amplitude of the fringe may show a crest or a trough, or some intermediate value which is referred to as the phase angle. It is almost true to say that with a set of fringes of given

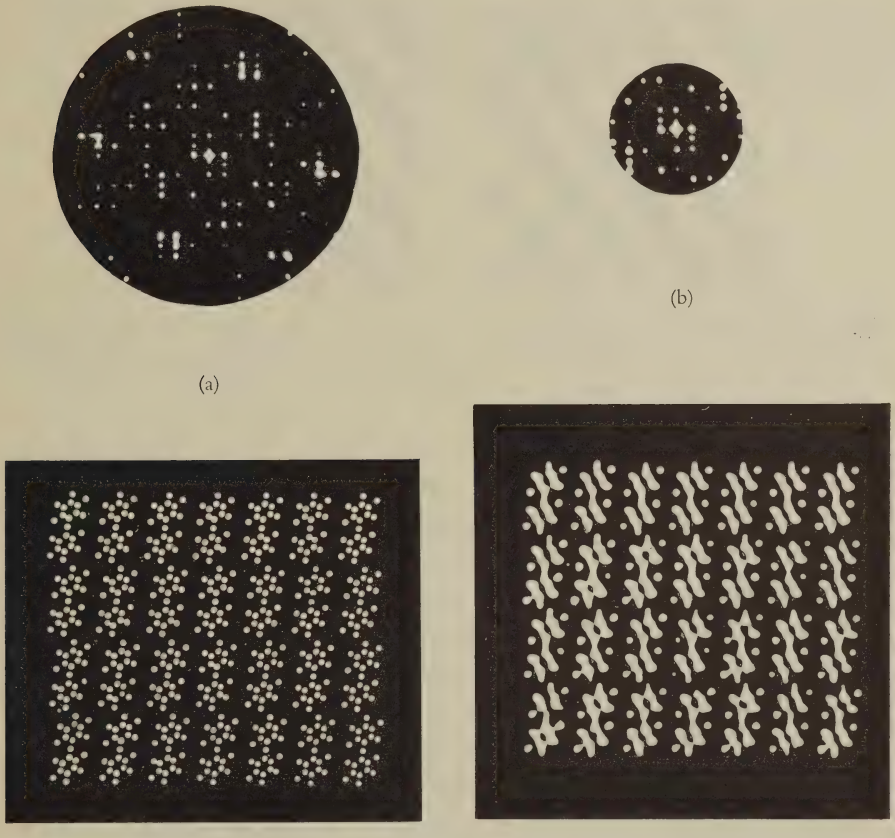

Fig. 4. (*a*) Image of hexamethyl benzene (*bottom*) reconstituted by recombination of the fringes produced by the X-ray diffraction pattern (*top*). The recombination is done in an optical diffraction apparatus. (*b*) Lack of resolution of image (*a*) caused by covering the outer part of the diffraction pattern. (*By courtesy of Dr. C. A. Taylor.*)

Fig. 5. Set of fringes related by different phase angles to the origin at the top left-hand corner. The phase angle marks the distance of the wave crest from the origin in degrees, one complete wavelength being taken as 360°.

amplitude, as shown in Fig. 5, an infinite number of different images can be generated, depending on the choice of phase for each of the fringes. By itself, the X-ray pattern tells only about the amplitude, but not about the phase angles of the fringes to be generated by each pair of spots, so that half the information needed for production of the image is missing.

The phase problem can be solved by the preparation of isomorphous crystalline compounds, one containing the protein alone, another containing the protein with a heavy atom, such as mercury, attached to the protein in some definite position, say 1, and yet another containing the protein with a heavy atom attached to a different position, say 2 (Fig. 6)[3–6].

The presence of heavy atoms produces measurable changes in the intensities of the diffraction pattern which allow information to be gathered about the values of the phases. This is done in the following way. From the difference in amplitude in the presence or absence of the heavy atom, the distance of the wave-crest from the heavy atom can be determined for each

set of fringes, thus giving the magnitude of the phase angle, with the heavy atom serving as a common origin. Unfortunately, this still leaves an ambiguity of sign, since we cannot tell whether the phase angle is to be measured in the forward or backward direction, but by examining the diffraction pattern from the second heavy atom compound, giving the distance of each wave-crest from heavy atom No. 2, the ambiguity can be resolved, provided that the vector H_1–H_2 is also known. The determination of that vector is one of the vital and often difficult steps in the X-ray analysis[7,8]. As long as the number of heavy atoms associated with each protein molecule is not too large, it can be overcome by calculation of the Fourier series[9] indicated in Fig. 6d.

Having solved the phase problem, we now have to consider the formation of the image. In simpler structures the atomic positions can often be found from two-dimensional representations projected on two mutually perpen-

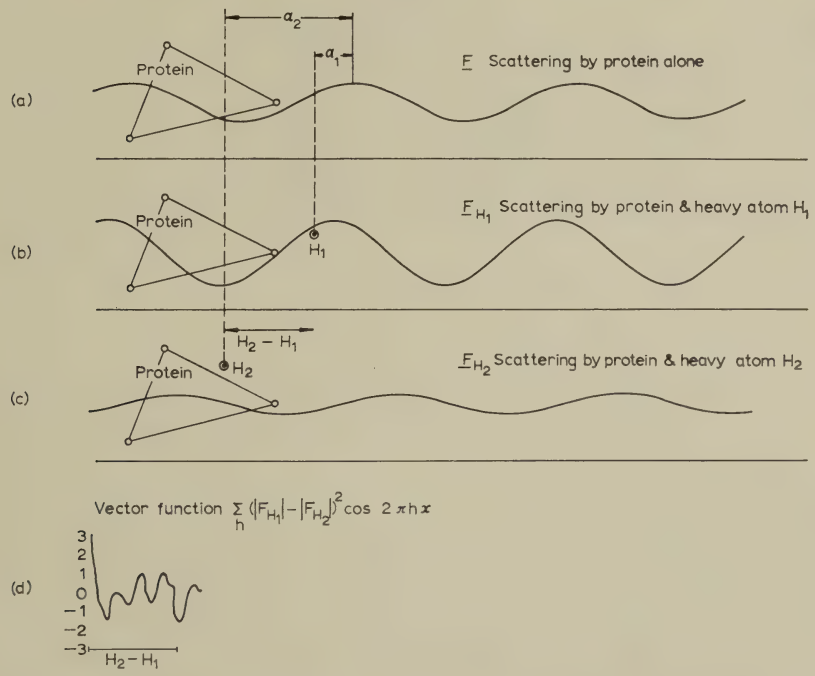

Fig. 6. (*a*) Diffraction by triangle representing a protein molecule. (*b*) and (*c*) Change in amplitude and phase of the diffracted wave caused by presence of heavy atoms H_1 and H_2. (*d*) Vector function devised by M. G. Rossmann. The Fourier sum shows a marked dip at a distance from the origin which corresponds to the vector H_1–H_2. Normally this function would be evaluated in three dimensions.

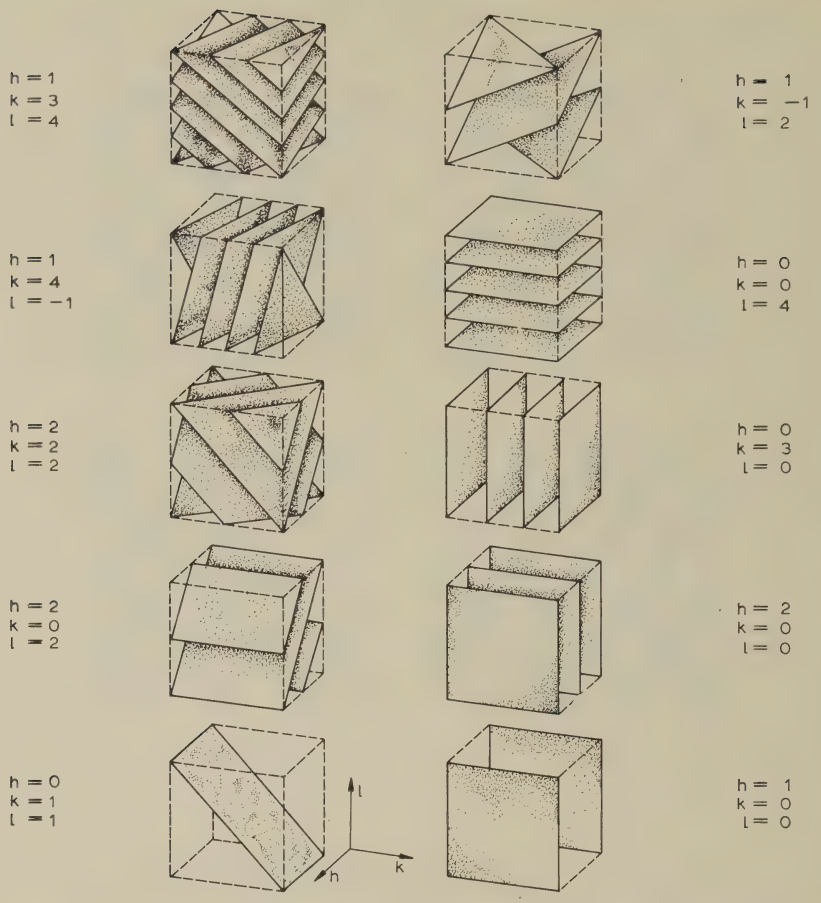

Fig. 7. Set of three-dimensional fringes used to build up image of the electron density. (*Reproduced, by permission, from W. de Beauclair, «Verfahren und Geräte zur mehrdimensionalen Fouriersynthese», Akademie-Verlag, Berlin, 1949.*)

dicular planes, but in proteins a three-dimensional image is essential. This can be attained by making use of the three-dimensional nature of the diffraction pattern. Fig. 2 can be regarded as a section through a sphere which is filled with layer after layer of diffracted spots. When these are recorded, each pair of symmetrically related spots can be made to generate a set of three-dimensional fringes, and these are then superimposed to build up the image of the protein (Fig. 7). The image is represented in the form of a series of sections through the molecule, rather like a set of microtome sections through a tissue, only on a thousand times smaller scale (Fig. 8).

Finally the question of resolution has to be faced. The resolving power of

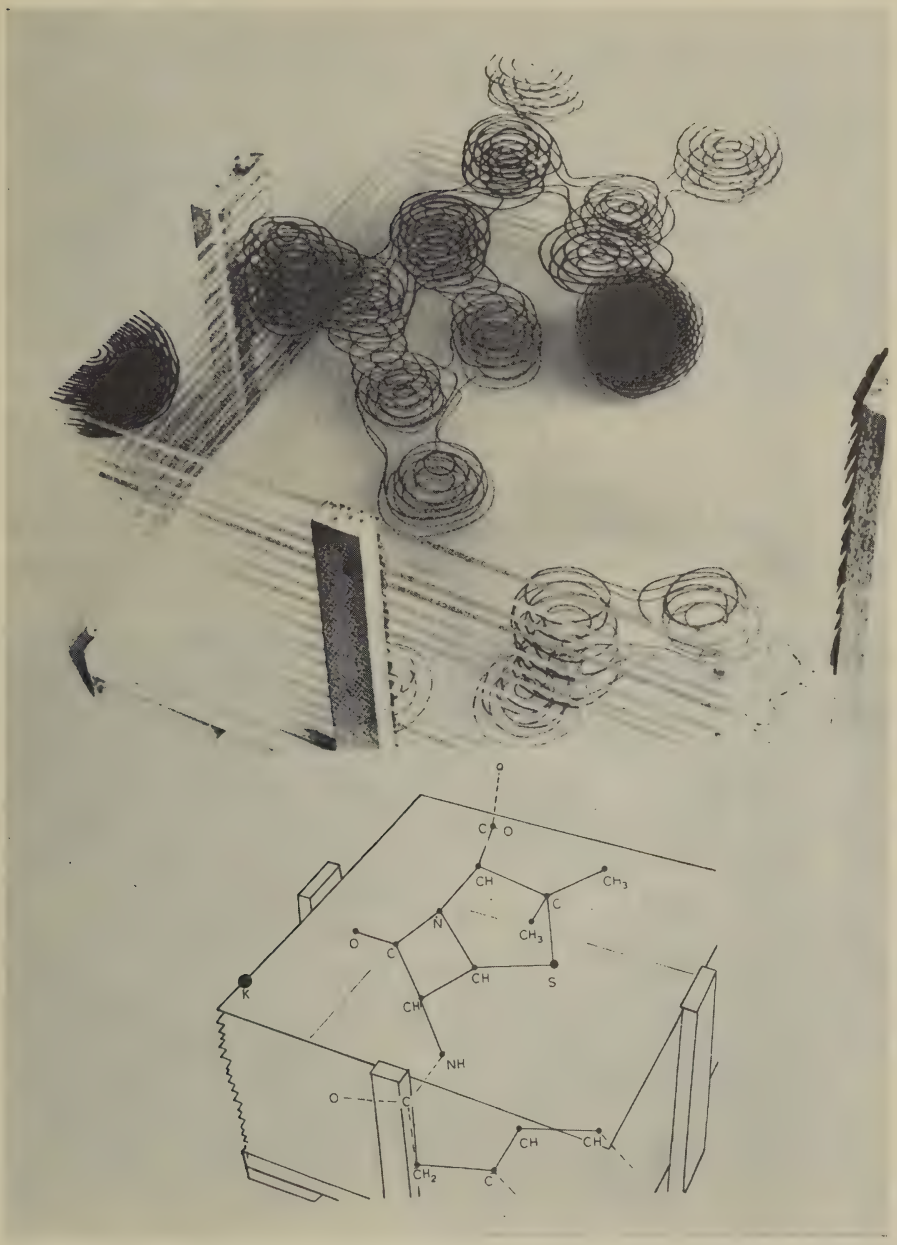

Fig. 8. Three-dimensional representation of the electron density distribution in penicillin, with resolution sufficient to separate individual atoms. (*Reproduced, by permission, from D. Crowfoot* et al., *Princeton University Press*).

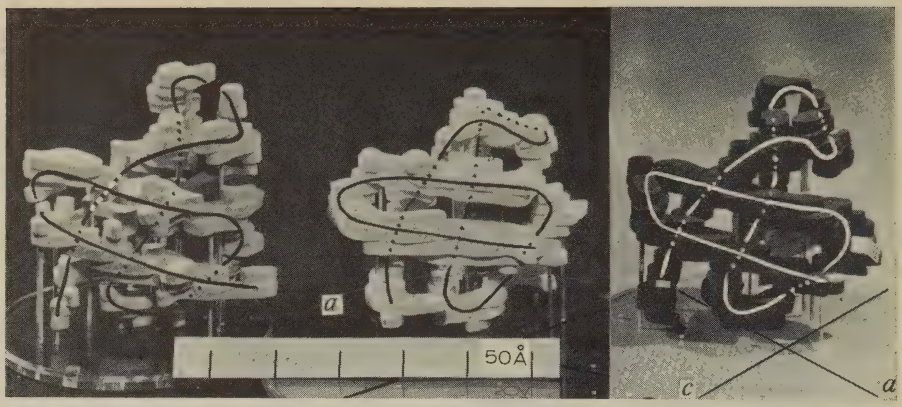

Fig. 9. Comparison of myoglobin (*a*) with the white and black units of haemoglobin (*b*) and (*c*).

the image is roughly equal to shortest wavelength of the fringes used in building it up, and that wavelength stands in a reciprocal relation to the angle which the corresponding diffracted ray makes with the incident X-ray beam (Fig. 3). If the image is built up from fringes corresponding to only part of the diffraction pattern, the resolution is impaired (Fig. 4b). In the X-ray diffraction patterns of protein crystals, the number of spots runs into tens of thousands. These all have to be measured in several isomorphous compounds, corrected by various geometric factors and finally used to build up an image by the superposition of tens of thousands of fringes. For instance, the calculation of a three-dimensional image of myoglobin at 2 Å resolution involved the recording and measuring of about a quarter of a million spots, and in the final calculation about 5×10^9 figures had to be added or subtracted[10]. Clearly this would have been impossible before the advent of high-speed computers, and we have in fact been very fortunate, because the development of computers has always just kept in step with the expanding needs of our X-ray analyses.

Function and structure of haemoglobin

Vertebrate haemoglobin is a protein of molecular weight 64,500. Four of its 10,000 atoms are iron atoms which are combined with protoporphyrin to form 4 haem groups. The remaining atoms are in four separate polypeptide chains, each containing just over 140 amino acid residues. In human haemo-

globin their sequence is now known[11,12]. In horse haemoglobin it has been partly elucidated and differs from the human form in only a small number of residues[13,14].

Haemoglobin acts as a carrier of oxygen from the lungs to the tissues and of carbon dioxide back to the lungs. The four iron atoms are in the ferrous state, and each is capable of combining reversibly with one molecule of oxygen without itself becoming oxidized in the process. The four iron atoms interact in a physiologically advantageous way, so that the combination of any one of them with oxygen increases the rate of combination with oxygen of its partners. A similar interaction takes place when the oxygen is given up. Carbon dioxide is not carried by the iron atoms directly, but its uptake by the red cells or the serum is facilitated by the disappearance of an acid group from each quarter molecule of haemoglobin when it has given up its oxygen. Conversely the presence of acid in the tissues speeds the liberation of oxygen by haemoglobin[15]. The haemoglobin molecule may therefore be regarded as an enzyme with two functions and several active sites,

Fig. 10. Two pairs of chains symmetrically related by the dyad axis. The arrow shows how one pair is placed over the other to assemble the complete molecule.

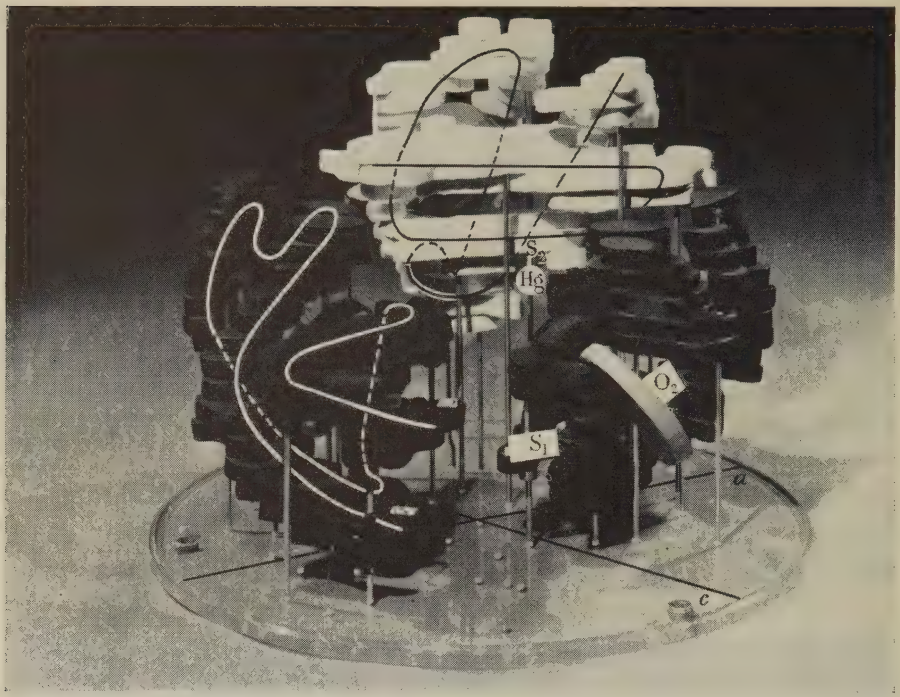

Fig. 11. Partly assembled molecule showing two black chains and one white. The *white ball* indicates a mercury atom attached to one of the «hidden» cysteines (S_2). S_1 indicates one of the reactive cysteines.

which interact in a complex and sophisticated manner. The explanation of this behaviour is one of the main objects of our research.

We have carried out three-dimensional X-ray analyses of the oxyhaemoglobin of horse and of the reduced haemoglobin of man. The resolution so far attained is 5.5 Å, which is sufficient to show the course of the polypeptide chains and the positions of the haem groups, but does not allow individual amino-acid residues to be seen. Nevertheless the results show that the reduced and the oxygenated forms differ markedly in structure, and they suggest that a molecular rearrangement accompanies the reaction of haemoglobin with oxygen.

The structure of horse oxyhaemoglobin has been published some time ago and I need merely remind you of its main features[16,17,18]. In agreement with the chemical evidence, the electron density maps show four haem groups and four separate chains which are identical in pairs. They are very similar in structure, and each chain bears a strong resemblance to sperm whale

myoglobin[10] whose molecular architecture will form the subject of Dr. Kendrew's discourse. He will show you that its single polypeptide chain is made up of eight helical stretches interrupted by corners or non-helical regions, and that the N-terminal end of the chain is at the bottom left (Fig. 9).

The haemoglobin molecule is assembled by first matching each chain with its symmetrically related partner (Fig. 10), then inverting the pair of white chains and placing it over the top of the pair of black ones (Fig. 11). The resulting arrangement is tetrahedral, the four subunits forming a compact spheroidal molecule with the haem groups arranged in separate pockets on the surface of the molecule (Figs. 12 and 13). The wide distances separating the haem groups was perhaps the greatest surprise the structure presented to us, for one would have expected the chemical interaction between them to be due to their close proximity. As it stands, the structure of oxyhaemoglobin leaves its physiological properties unexplained.

The oxygen-free form of haemoglobin, called somewhat inappropriately reduced haemoglobin, has long been known to differ from oxyhaemoglobin

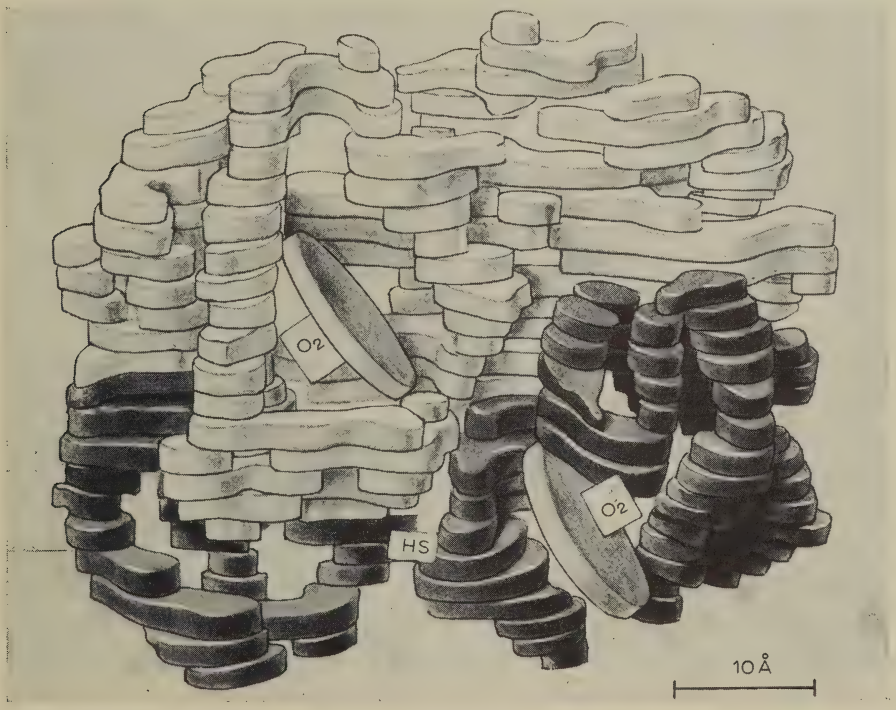

Fig. 12. Complete haemoglobin molecule. The haem groups are indicated by grey disks.

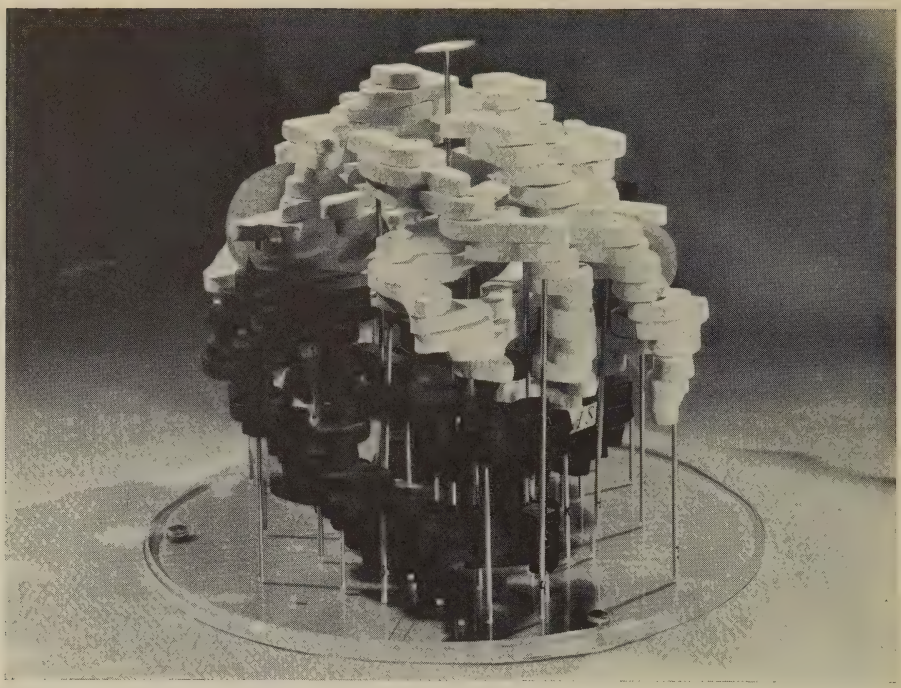

Fig. 13. Haemoglobin molecule viewed in another direction.

in its solubility, crystal structure,[19–21] and other properties, which suggested that the explanation should perhaps be sought in a structural rearrangement between the two forms. Unfortunately, reduced haemoglobin of horse, crystallizes in a form unsuitable for detailed X-ray analysis, so that we turned to human haemoglobin which is more amenable. This choice leaves a gap in the argument, because the structure of human oxyhaemoglobin is still unknown and might conceivably differ from that of horse oxyhaemoglobin in the same manner as the human reduced form. In view of the close similarity between the amino acid sequences of the two species, this seems unlikely, but obviously the point remains to be proved. (See note on p. 673.)

So far the structure of human reduced haemoglobin is based on the X-ray analysis of only a small number of isomorphous heavy atom derivatives, as compared to the six used for horse oxyhaemoglobin, and for technical reasons these particular derivatives are insufficient to determine the phase angle accurately[22].

Despite these imperfections, several features stand out clearly. The molecule is made up of four subunits which appear to be very similar in structure

to those found in horse oxyhaemoglobin (Fig. 14), but there is a striking rearrangement of the two black subunits, involving an increase in the distance between symmetrically related features by over 7 Å. The relative arrangement of the white subunits is unchanged (Fig. 15).

Chemically, the white units are known as the α- and the black as the β-chains[23]. The rearrangement of the two β-chains in reduced haemoglobin must of course have come about as the result of some structural rearrangement within them, or possibly at the points of contact between the α- and β-chains. Small structural changes cannot be distinguished at the present resolution, but it should be possible to detect any major change, for instance in the angles between the helical segments. However, these angles appear to be the same, within the limits of error, in human reduced and in horse oxyhaemoglobin, and whatever structural changes take place within the individual chains must be too small to be detected at the present resolution.

We may hope that that interaction, and the acid shift on which the res-

Fig. 14. Comparison of black β-chains of horse oxyhaemoglobin with the corresponding ones of the human reduced form. (Note the helical regions marked G and H.)

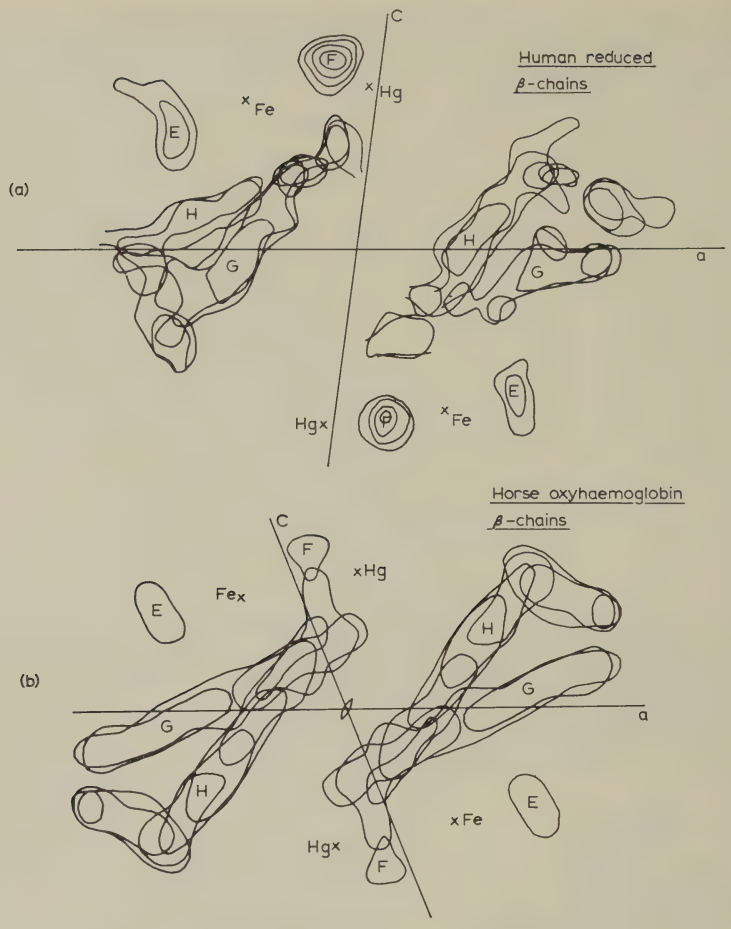

Fig. 15. (*a*) Projection of the helical regions G and H, together with the positions of the iron and mercury atoms (attached to cysteines) and sections through the helices E and F, in human reduced haemoglobin. (*b*) Same for horse oxyhaemoglobin. (*Fig. 15 continued on pp. 669 and 670.*)

piratory functions of haemoglobin depend, will eventually find their explanation in terms of the structural changes of which these new results have just given us a first glimpse, but it may well be necessary to solve the structure of at least one of the two forms in atomic detail. Due to the enormous amount of labour involved this may take some time, but not much, perhaps, compared to the 22 years needed for the initial analysis.

The discovery of a marked structural change accompanying the reaction of haemoglobin with oxygen suggests that there may be other enzymes

which alter their structure on combination with their substrate, and that this may perhaps be an important factor in certain mechanisms of enzymatic catalysis.

Please forgive me for presenting, on such a great occasion, results which are still in the making, but the glaring sunlight of certain knowledge is dull and one feels most exhilarated by the twilight and expectancy of the dawn. Thank you again for your magnificent award.

Acknowledgements

I have already alluded to the great part played by Sir Lawrence Bragg in supporting this work, but I also owe an immense debt for the many ideas he contributed and for his astute criticism. Sir Harold Himsworth, as Secretary of the Medical Research Council, made sure that we never lacked any of the

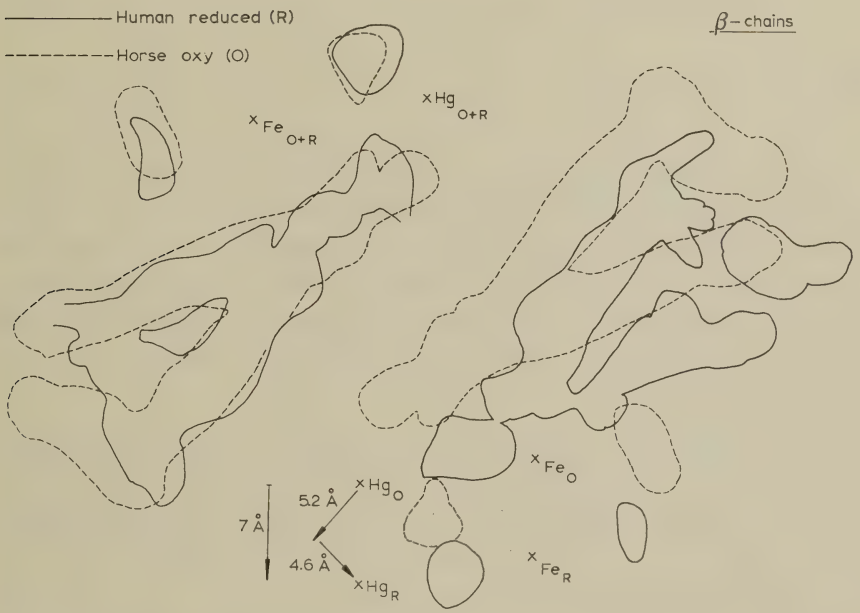

Fig. 15 (*ctd.*): (*c*) Superposition of (*a*) and (*b*) in outline, showing difference in structure between the two forms. At the *top left*, one member of each pair has been superimposed so as to bring the iron and mercury positions into coincidence. The *bottom right* shows the shift of the two chains relative to each other.

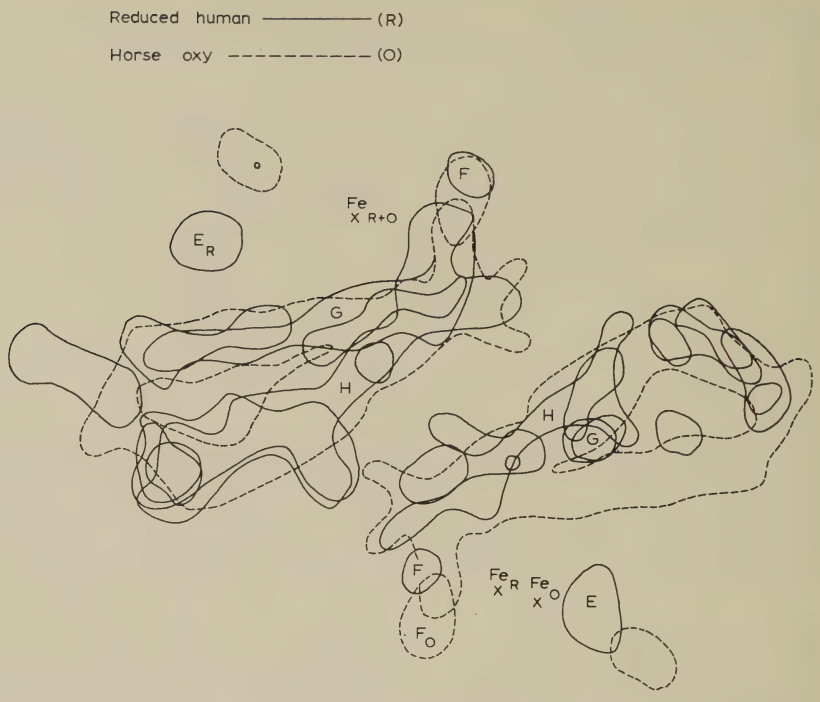

Fig. 15 (*ctd.*): (*d*) Superposition of white chains, showing little change of structure.

funds or equipment needed for our work. Without his consistent support, research on the required scale could not have been done. Dr. G. R. Pomerat, as one of the Directors of the Rockefeller Foundation, helped us to cover any additional expenditure which the Medical Research Council had difficulty in meeting. Moreover, the Rockefeller Foundation supported me in the early years; without them the work would probably not have got beyond its initial stages.

To the many others who helped me I should like to acknowledge my debt in the accompanying table, which records their contributions with literature references wherever possible. The long list makes me realize how much I owe to my colleagues. I hope that they will accept it as a token of my gratitude.

Contributors to the Work on the Structure of Haemoglobin

Name	*Contribution*	*Reference*
David M. Blow	Development of experimental and theoretical methods for the determination of phase angles by multiple isomorphous replacement.	5
D. A. G. Broad	Design and construction of rotating anode tubes used to produce the high-intensity X-ray beams essential for X-ray diffraction work on proteins. He also designed many other instruments used in this work.	24
Ann F. Cullis	acted as my research assistant during the crucial years when the three-dimensional X-ray analysis was being done.	16, 17, 18, 25
Howard M. Dintzis	introduced new methods for attaching heavy atoms to haemoglobin.	25
John A. L. Fasham	reconstructed and improved the microdensitometer used in measuring the intensities of the X-ray reflexions.	
Michael Fuller	maintained the rotating anode tubes.	
David W. Green	did some of the early work on reduced human haemoglobin and helped me with the first Fourier projections of horse oxyhaemoglobin.	4, 21
L. G. Hayward	made the rotating anode tubes and many other instruments used in the work.	
Eric R. Howells	helped me to interpret the X-ray pattern of one of the forms of horse haemoglobin. He also did much other useful work on the structure.	27, 28
David J. E. Ingram	determined the inclination of the haem groups by electron spin resonance.	26
Vernon M. Ingram	prepared the first crystalline heavy atom derivatives of haemoglobin.	4
Ann Jury	worked as my research assistant in 1959/1960.	
Christian K. Møller	worked on the infrared dichroism of haemoglobin.	
Hilary Muirhead	developed a computer programme for calculating the phase angles in horse oxyhaemo-	16–18, 22

Name	*Contribution*	*Reference*
	globin. She also did most of the work on reduced human haemoglobin reported here.	
M. Murayama	made one of the doubly substituted heavy atom derivatives and also improved our chemical methods.	
A. C. T. North (Royal Institution, London)	determined the absolute intensity of the X-ray reflexions, and the relative intensities of all the stronger reflexions by counter spectrometer methods.	16–18
D. C. Phillips (Royal Institution, London)	measured the absolute intensities of reflections from haemoglobin cystals.	
Michael G. Rossmann	developed the mathematical methods used for determination and refinement of the heavy atom parameters and also devised most of the computer programmes used in this work.	9, 16–18
Larry L. Steinrauf	tried to develop new methods of introducing heavy atoms into haemoglobin.	
Ian F. Trotter	did much of the early work on reduced human haemoglobin.	21
Georg Will	took the first set of three-dimensional X-ray photographs of horse haemoglobin and worked out the geometric correction factors.	16

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Note added December 1963. When I received the Nobel Prize a year ago, Miss Muirhead and I had just arrived at the first tentative solution of the structure of human reduced haemoglobin. Since then we have obtained a much clearer set of electron density maps which confirmed the differences between the structures of horse oxy and human reduced haemoglobin just described. However, we were unable to decide whether these were due to the oxygenation reaction, as we believed, or merely to the difference in species[22].

Recently I discovered a new crystalline form of horse reduced haemoglobin and found its structure to be similar to that of human reduced, rather than to that of horse oxyhaemoglobin. Thus the rearrangement of the «black» chains is really due to the oxygenation reaction: a most remarkable effect.

Biography

Max Ferdinand Perutz was born in Vienna on May 19th, 1914. Both his parents, Hugo Perutz and Dely Goldschmidt, came from families of textile manufacturers who had made their fortune in the 19th century by the introduction of mechanical spinning and weaving into the Austrian monarchy. He was sent to school at the Theresianum, a grammar school derived from an officers' academy of the days of the empress Maria Theresia, and his parents suggested that he should study law in preparation for entering the family business. However, a good schoolmaster awakened his interest in chemistry, and he had no difficulty in persuading his parents to let him study the subject of his choice.

In 1932, he entered Vienna University, where he, in his own words, «wasted five semesters in an exacting course of inorganic analysis». His curiosity was aroused, however, by organic chemistry, and especially by a course of organic biochemistry, given by F. von Wessely, in which Sir F. G. Hopkins' work at Cambridge was mentioned. It was here that Perutz decided that Cambridge was the place where he wanted to work for his Ph.D. thesis. With financial help from his father he became a research student at the Cavendish Laboratory in Cambridge under J. D. Bernal in September 1936, and he has stayed at Cambridge ever since.

After Hitler's invasion in Austria and Czechoslovakia, the family business was expropriated, his parents became refugees, and his own funds were soon exhausted. He was saved by being appointed research assistant to Sir Lawrence Bragg, under a grant from the Rockefeller Foundation, on January 1st, 1939. The grant continued, with various interruptions due to the war, until 1945, when Perutz was given an Imperial Chemical Industries Research Fellowship. In October 1947, he was made head of the newly constituted Medical Research Council Unit for Molecular Biology, with J. C. Kendrew representing its entire staff. He continued holding this post until he was made Chairman of the Medical Research Council Laboratory of Molecular Biology, in March 1962. His collaboration with Sir Lawrence Bragg has continued through all these years.

The scientific work of Perutz on the structure of haemoglobin started as a result of a conversation with F. Haurowitz in Prague, in September 1937. G. S. Adair made him the first crystals of horse haemoglobin, and Bernal and I. Fankuchen showed him how to take X-ray pictures and how to interpret them. Early in 1938, Bernal, Fankuchen, and Perutz [*Nature*, 141 (1938) 523] published a joint paper on X-ray diffraction from crystals of haemoglobin and chymotrypsin. The chymotrypsin crystals were twinned and therefore difficult to work with, and so Perutz continued with haemoglobin. D. Keilin, then Professor of Biology and Parasitology at Cambridge, soon became interested in the work and provided Perutz and his colleagues with the biochemical laboratory facilities which they lacked at the Cavendish. Thus from 1938 until the early fifties the protein chemistry was done at Keilin's Molteno Institute and the X-ray work at the Cavendish, with Perutz busily bridging the gap between biology and physics on his bicycle. The rest of the story is well-known and forms the subject of his Nobel discourse.

Perutz has persued one sideline concerned with glaciers, studying their crystal texture and mechanism of flow, but this was mainly an excuse for working in the mountains: he is a keen mountaineer, his other recreations being walking, skiing and gardening. Scientifically, his overwhelming interest lies on the side of molecular biology. He is grateful for having had the good fortune of being joined by colleagues of great ability, several of whom have now been honoured with the Nobel Prize at the same time as Perutz himself. Kendrew came in 1946, Crick in 1948, and Watson arrived as a visitor in 1951. Recently F. Sanger, who received the Nobel Prize in 1958, also joined forces with them. Perutz is extremely happy at the generous recognition given by the Swedish Academy of Sciences and the Royal Karolinska Institute to their great common adventures and hopes that it will spur them to new endeavours.

Perutz, who is a Fellow of the Royal Society, was made Companion of the British Empire in 1962. He is also an honorary member of the American Academy of Arts and Sciences.

In 1942, Perutz married Gisela Peiser. They have two children, Vivien (b. 1944) and Robin (b. 1949).

John C. Kendrew

Myoglobin and the structure of proteins

Nobel Lecture, December 11, 1962

When I first became interested in the question of solving the structure of proteins, during the latter part of the Second World War, I had no doubt that this problem above all others deserved the attention of anyone concerned with fundamental aspects of biology. Had my interests been awakened a few years later I would, no doubt, have recognized that there were in fact two such basic unanswered questions, the structure of proteins and the structure of nucleic acids. As events have turned out, the second question was posed later and answered sooner. For me in the early 1940's, however, there seemed to be only one question uniquely qualified to engage the interest of anyone wishing to apply the disciplines of physics and chemistry to the problems of biology. It also seemed that the only technique offering any chance of success in determining the structures of molecules so large and complex as proteins was that of X-ray crystallography. Looking back on that time it occurs to me that my own almost total ignorance of this method was fortunate, in that it concealed from me the extent to which contemporary X-ray crystallographic techniques fell short of what was needed to solve the structures of molecules containing thousands of atoms; it was indeed a case of ignorance being bliss. For a number of years, this situation persisted – many roads were explored, but none of them seemed to offer real hope of a definitive solution – until my colleague Dr. Max Perutz showed that the method of isomorphous replacement, until then applied rather rarely in crystallography generally, and never in the field under discussion, was in fact ideally suited to the protein problem. His first successful application of this method to the haemoglobin structure provided the basis of all subsequent work in the field, my own included. Perutz has included an account of the method in his lecture, and in the present discussion I shall therefore refer to questions of methodology only in so far as they have special relevance to my own work.

As I have indicated, my choice of problem and of method seemed straightforward. The choice of material was not so simple. One looked for a protein of low molecular weight, easily prepared in quantity, readily crystallized,

and not already being studied by X-ray methods elsewhere. Myoglobin seemed to satisfy these criteria, and had the additional advantages of being closely related to haemoglobin, already the object of Perutz's attention for many years, and of sharing with haemoglobin a most important and interesting biological function, that of reversible combination with oxygen. As emerged more clearly later, myoglobin consists of a single polypeptide chain of about 150 amino acid residues, associated with a single haem group; its one-to-four relationship with haemoglobin already suggested in early days by a comparison of molecular weights, turned out to be not coincidental but a fundamental structural relationship, as has now been shown by comparing the molecular models of the two proteins. At the beginning, however, one was more concerned with practical problems which took a number of years to solve, than with hypothetical structural relationships.

First of all it was necessary to find some species whose myoglobin formed crystals suitable, both morphologically and structurally, to the purpose in hand; the search for this took us far and wide, through the world and through the animal kingdom, and eventually led us to the choice of the sperm whale, *Physeter catodon*, our material coming from Peru or from the Antarctic, with some close runners-up including the myoglobin of the common seal, whose structure is now being studied by Dr. Helen Scouloudi at the Royal Institution in London. Once the method of isomorphous replacement had been shown to be capable in principle of solving the structure, one was faced with the task of attaching a small number of very heavy atoms at well-defined sites to each protein molecule in the crystal. Myoglobin lacks the sulphydryl groups whose presence in haemoglobin was so successfully exploited by Perutz and Ingram for the attachment of mercurial reagents; we had to look for other ways, and our attempts to use the unique haem group for the attachment of ligands which contained heavy atoms having proved for the most part unsuccessful (our ligands were always rapidly ejected by even very small traces of oxygen which were almost impossible to exclude), we were thrown back to a more empirical approach. This consisted in crystallizing myoglobin in the presence of metallic ions and then seeing whether any changes in the X-ray pattern could be detected; further analysis was required to determine whether, as we desired, substitution had taken place at a single site. In the absence of any sound foundation of theory, it was necessary to examine a very large number of possible ligands – several hundreds – before two or three were found which satisfied all the rather rigid criteria. Such laboriously empirical procedures are still forced upon all

workers in this field, and very drastically limit the exploitation of the isomorphous replacement method. A rational and generally applicable solution to this problem still awaits discovery, and would do more than any other single factor to open up the field.

General strategy of the analysis

Turning now to the strategy actually adopted for the solution of the structure, we may remember that Perutz's first application of the isomorphous replacement method in haemoglobin, as well as our own in myoglobin, had been to produce a two-dimensional projection of the structure. For such a projection the number of X-ray reflexions required is fairly small, and the solution of the phase problem is simple; even with a single isomorphous replacement the results are unambiguous. But the amount of structural information which could be derived from a projection was almost nil, owing to the high degree of overlapping of the elements of so complex a structure. It was immediately clear that to exploit the method it had to be applied in three dimensions, to produce a spatial representation of the electron density throughout the crystal. This involved the study of a much larger number of reflexions and the calculation of general phases, and required, for an unambiguous solution, the comparison of several heavy-atom derivatives substituted in different parts of the molecule.

The whole diffraction pattern of a myoglobin crystal consists of at least 25,000 reflexions. In 1955, when the three-dimensional work began, no computers existed which were fast enough to calculate Fourier syntheses containing so many terms; besides, the method was unproved and it seemed advisable to test it first on a smaller sample of data.

We may regard a typical X-ray photograph of a myoglobin crystal (Fig. 1) as a two-dimensional section through a three-dimensional array of reflexions; each reflexion corresponds to a single Fourier component, and the whole structure can be reconstructed by using all the components as terms of a Fourier synthesis. As Perutz has indicated in his lecture, the components of higher frequency (higher harmonics), which are responsible for filling in the fine details of the structure, lie to the outside of the pattern. Thus one can obtain a rendering of the molecule at low resolution by using simply those reflexions within a spherical surface at the centre of the pattern. By doubling the radius of the sphere (which now encloses eight times as many reflexions)

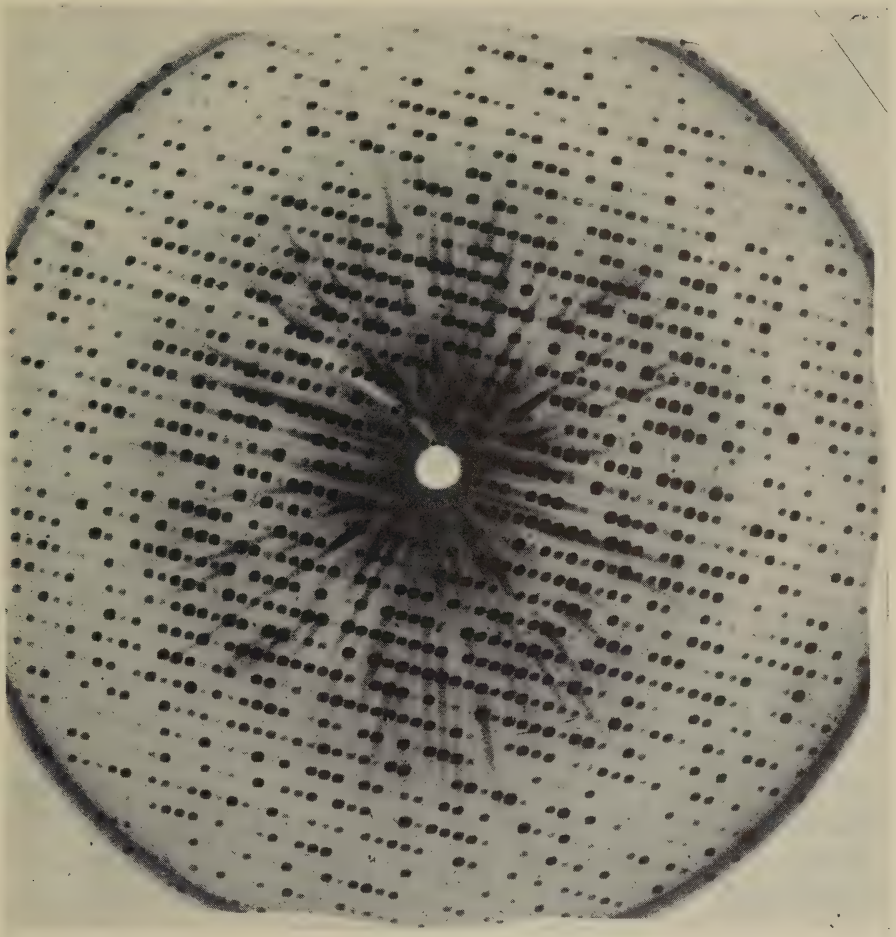

Fig. 1. X-ray precession photograph of a myoglobin crystal.

we double the resolution of the density distribution. We actually decided to undertake the solution of the structure in three stages; the first, completed in 1957, involved 400 reflexions and gave a resolution of 6 Å; the second (1959) included nearly 10,000 reflexions and gave a resolution of 2 Å; the third (not yet complete) includes all the observable reflexions – about 25,000 – and gives a resolution of 1.4 Å. It may be recalled that polypeptide chains pack together at centre-to-centre distances of 5 to 10 Å; that atoms (other than hydrogen) of neighbouring groups in Van der Waals contact, or brought together by hydrogen bonds or charge interactions, lie 2.8 to 4 Å apart; and that the separation between covalently bonded atoms is 1 to 1.5

Å. It follows that the three stages chosen would be expected to separate polypeptide chains, groups of atoms, and individual atoms, respectively. The third stage, with its resolving power of 1.4 Å, should only just distinguish neighbouring covalently bonded atoms, but this is as far as the analysis can go because beyond this point the diffraction pattern fades away. This limit represents a lower degree of order than is usual in crystals of molecules of low or moderate complexity; in fact myoglobin crystals possess a higher degree of order than do those of almost all other proteins, and this was an additional reason for my choice of this protein for analysis.

Before proceeding to describe the results of the three stages of the analysis, it will be convenient to revert to the question of computers. As will be evident from what follows, the amount of useful structural information obtainable increases rapidly with the resolving power. Indeed it seems probable that for most proteins the dividend obtained from high resolution would be even greater, for it has emerged that the helix content of myoglobin (75%) is a good deal higher than that of most other proteins, and the identification of structural features in myoglobin at less than atomic resolution was greatly dependent on the presence of many helical segments of polypeptide chain, readily identifiable even at 6 Å resolution and already at 2 Å resolution providing well-defined take-off points for side chains, often enabling these to be identified even though their individual atoms could not be distinguished. But, as already indicated, the amount of computation required increases very rapidly with the resolving power. Even at the first stage of the analysis we made use of an electronic computer, EDSAC I, which though small and slow by modern standards was at the time one of the very few such instruments in operation in the world; it is significant that these early Fourier syntheses of the myoglobin data were, to the best of my belief, the first crystallographic computations ever carried out on an electronic computer and initiated a practice which later (and incidentally after a time lag of several years) became universal among crystallographers. At each stage of the myoglobin analysis the computers employed were among the most rapid available at the time, and we are now using very fast and large computers such as EDSAC II and IBM 7090; most proteins are larger than myoglobin, and will need even bigger computers. There are also problems of data collection and data handling. In the myoglobin analysis the data for the 6 Å and 2 Å stages were mostly collected by conventional photographic methods; but at the 2 Å stage the solution of the phase problem for 9,600 reflexions involved the densitometry of some quarter of a million spots in all,

from different heavy-atom derivatives and exposures of different lengths. This represents something near the limit of the practicable, especially as we were aiming for, and achieved, a mean error of 2 to 4% in the determination of amplitude; personally I would not care to have to undertake such a task a second time. In any case, serious effects of radiation damage to the crystals make photographic techniques increasingly difficult if not impossible at the higher resolutions. Fortunately, the automatic diffractometer designed by my colleagues Drs. U. W. Arndt and D. C. Phillips became available just in time for the final stage of the work; with this apparatus the intensities of successive reflexions, measured with a proportional counter, are recorded on punched tape which can be fed direct into a high-speed computer. There is no doubt that automatic data-collecting equipment and very fast large computers will be highly desirable for all, and essential for most, X-ray studies of proteins.

Myoglobin at 6Å resolution

The three-dimensional electron density distribution in a crystal is most conveniently represented as a series of contour maps plotted on parallel transparent sheets; the function drawn in this way for myoglobin at 6 Å resolution is shown in Fig. 2. A cursory inspection of the map showed it to consist of a large number of rod-like segments, joined at the ends, and irregularly wandering through the structure; a single dense flattened disk in each molecule; and sundry connected regions of uniform density. These could be identified respectively with polypeptide chains, with the iron atom and its associated porphyrin ring, and with the liquid filling the interstices between neighbouring molecules. From the map it was possible to «dissect out» a single protein molecule, its boundaries being demarcated by the adjoining liquid; a scale model of this is shown in Fig. 3. For the most part the course of the single polypeptide chain could be followed as a continuous region of high density, but some ambiguities remained, especially at the irregular regions between two straight rods. The most striking features of the molecule were its irregularity and its total lack of symmetry; this made all the more remarkable the later finding by Perutz that each of the four sub-units of haemoglobin closely resembled the myoglobin molecule, in spite of wide differences in species and in amino acid composition.

As expected, it was not possible at 6 Å resolution to draw any conclusions

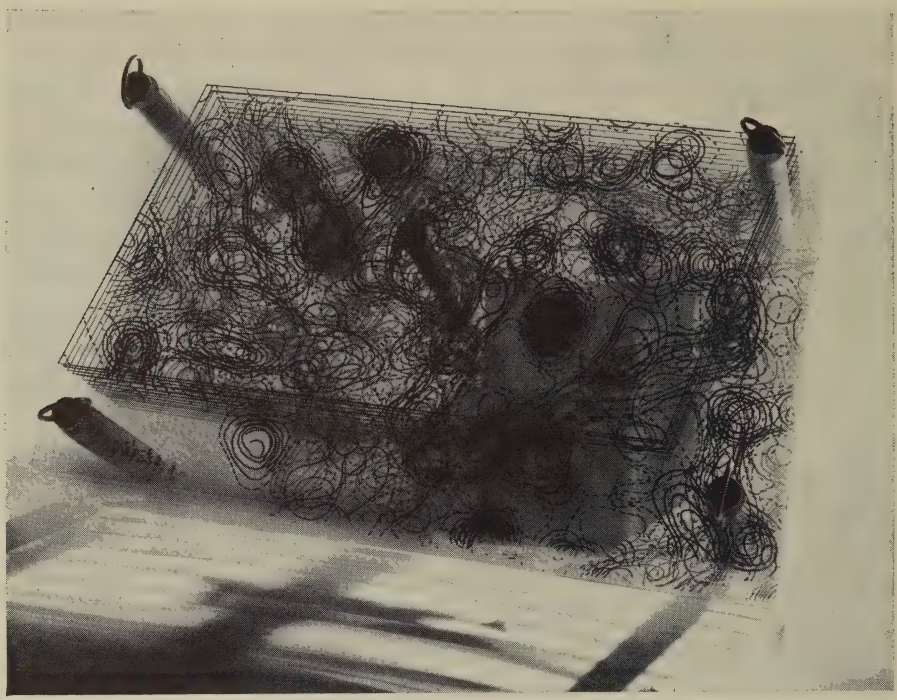

Fig. 2. Fourier synthesis of myoglobin at 6Å resolution.

regarding the nature of the folding of the popypeptide chain, or to see, let alone identify, side chains.

Myoglobin at 2Å resolution

To achieve a resolution of 2 Å it was necessary to determine the phases of nearly 10,000 reflexions, and then to compute a Fourier synthesis with the same number of terms. As already indicated, this task represented about the extreme limit of what is practicable by photographic techniques, and the Fourier synthesis itself (excluding preparatory computations of considerable bulk and complexity) required about 12 hours of continuous computation on a very fast machine (EDSAC II). The electron density function was calculated at about 100,000 points in the molecule, and was represented on the three-dimensional contour map shown in Fig. 4. In this photograph we are looking at the density distribution directly along the axis of one of the

Fig. 3. Model of the myoglobin molecule, derived from the 6Å Fourier synthesis. The haem group is a dark grey disk (*centre top*).

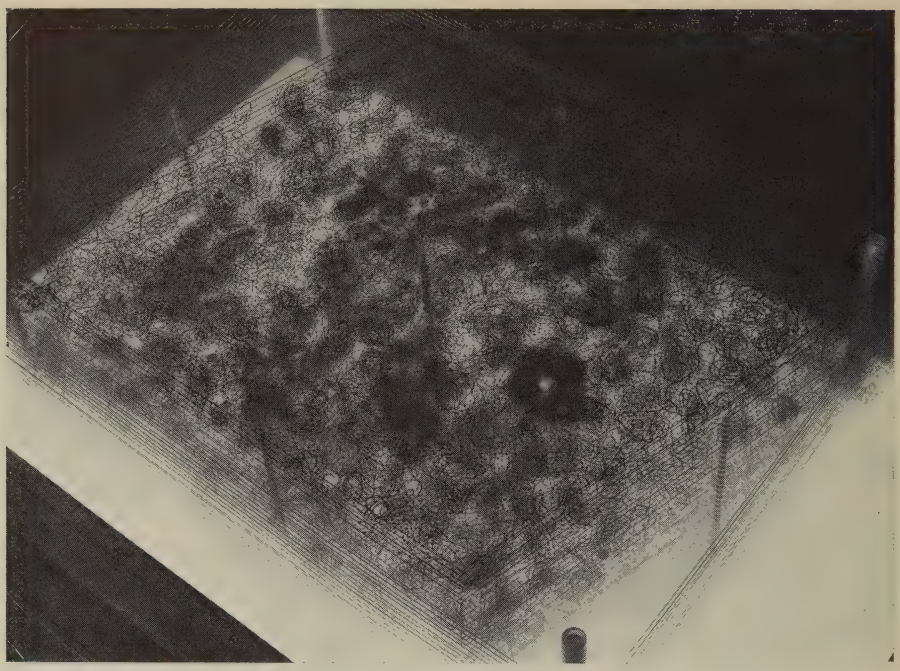

Fig. 4. Fourier synthesis of myoglobin at 2Å resolution, showing a helical segment of polypeptide chain end-on.

straight rod-like sections of polypeptide chain identified at low resolution; it will be seen that the rod has now developed into a straight hollow cylinder. Study of the density distribution on the surface of this (and other) cylinders showed that it fits the arrangement of atoms in the α-helix, postulated by Pauling & Corey in 1951 as the chain configuration in the so-called α-family of fibrous proteins; careful analysis of the density distribution, carried out on the computer, shows that the helical segments are nearly all precisely straight, and that their co-ordinates correspond to those given by Pauling & Corey within the limits of error of the analysis. Furthermore it is possible to see directly the orientation of each side chain relative to the atoms within the helix, and hence, from a knowledge of the absolute configuration of an L-amino acid, to show that all the helices are right-handed.

Another view (Fig. 5) of the contour map shows the haem group edge-on, now appearing as a flat disk with the iron atom at its centre. To our surprise we found that the iron atom lay more than $\frac{1}{4}$ Å out of the plane of the group; it was only later that we heard from Dr. Koenig at Johns Hopkins University that he had observed the same phenomenon in his structure anal-

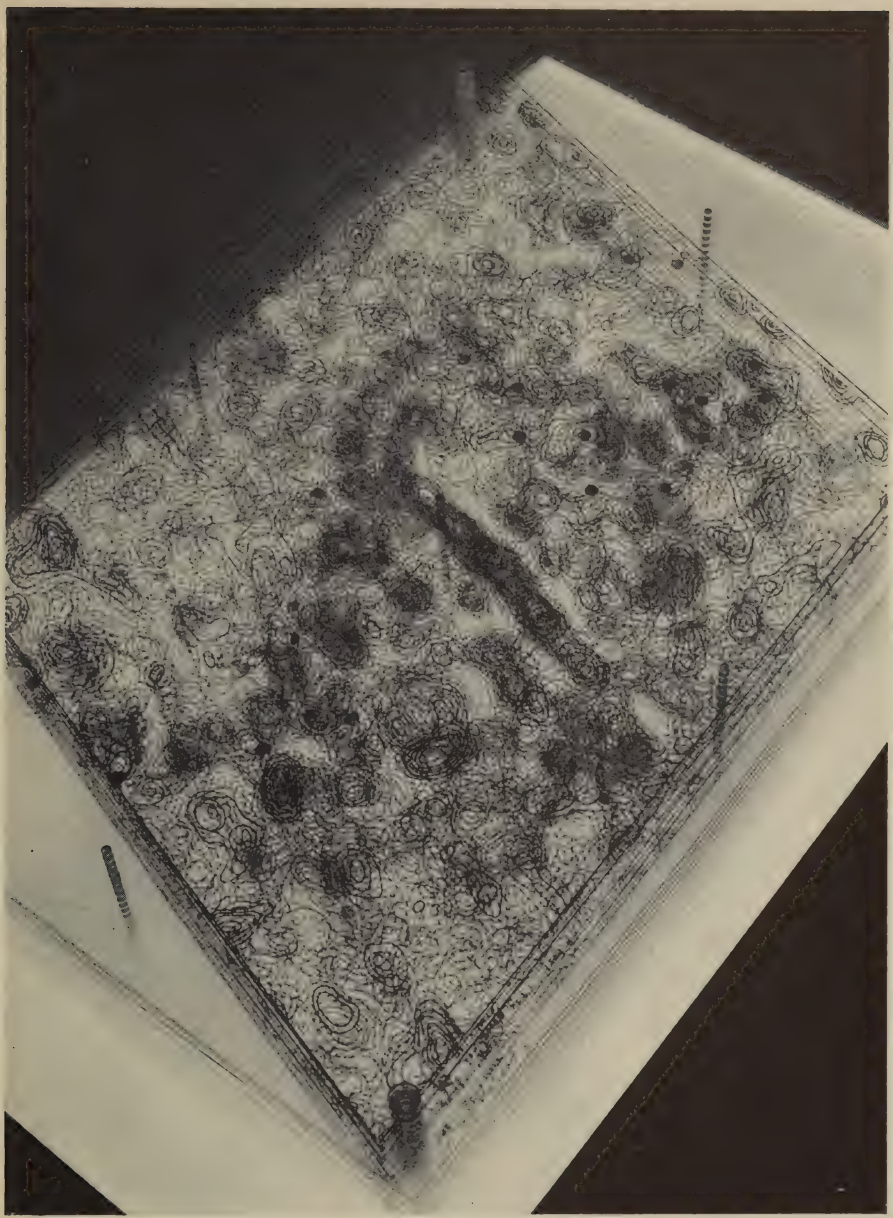

Fig. 5. Fourier synthesis of myoglobin at 2Å resolution, showing haem group edge-on.

ysis of haemin. We were also able to see that the iron atom was attached to one of the helical segments of polypeptide chain by a group which we were later able to identify as histidine – a striking confirmation of suggestions which had been made as much as thirty years earlier to the effect that histidine was the haem-linked group in haemoglobin and myoglobin.

In our preliminary publication about this Fourier synthesis in 1960, we pointed out that at a resolution of 2 Å, neighbouring covalently bonded atoms are not resolved, and gave it as our opinion that systematic identification of side chains would not be possible at this resolution. Events proved that we had been too pessimistic; by studying carefully the shapes of the lumps of density projecting at the proper intervals from the polypeptide

	Leu	Leu	Leu 5
	Phe	Phe	Phe 5
	Lys	Lys	Thr 3, Lys 2
	Ser	Ser	Ser 3, Thr 2
C1	His	His	His 5
	Pro	Pro	Pro 4
	Glu.C	Glu	Glu.C 5
	Thr	Thr	Thr 4
	Leu	Leu	Leu 4
	Glu	Glu	Ser 2, Thr 2
	Lys	Lys	Lys 3
CD1	Phe	Phe	Phe 5
	Asp	Asp	Asp 2
	Arg	Arg	Arg 3
	Phe	Phe	Phe 4
	Lys	Lys	Lys 3
	His	His	His 4
	Leu	Leu	Leu 2, Asp 2
	Lys	Lys	Lys 1
D1	Thr	Thr	Thr 5
	Glu.C	Glu	Glu 4
	Ala	Ala	Ala 5
	Glu.C	Glu	Glu 3
	Met	Met	Met 5
	Lys		Lys 4

Fig. 6. A comparison between chemical and X-ray evidence for part of the amino-acid sequence of myoglobin. (*First column*) tryptic peptides; (*second column*) chymotryptic peptides; (*third column*) X-ray evidence. Peptides are enclosed by brackets. In the third column, the figure gives the degree of confidence in the identification (5 = complete confidence, 1 = a guess).

chain we were often able to identify them unambiguously with one of the seventeen different types of side chain known, from the overall composition, to be present in the myoglobin molecule. We were able to seek confirmation and extension of our results from a quite different source. At the time when the myoglobin program was getting seriously under way I discussed with Drs. W. H. Stein and Stanford Moore at the Rockefeller Institute in New York the possibility that some member of their laboratory might undertake a determination of the complete amino acid sequence of myoglobin, using the methods originally employed by Sanger in his studies of insulin, and later developed and extended at the Rockefeller Institute for the analysis of ribonuclease. They were kind enough to arrange that Dr. Allen Edmundson, at that time a graduate student working in their laboratory under the supervision of Dr. C. H. W. Hirs, should undertake this task. By the time our 2 Å synthesis was available, Dr. Edmundson had studied most of the peptides obtained by tryptic digestion of myoglobin, determining their composition and in a few cases the sequence of residues within them. We found that, by laying his peptides along the partial and tentative sequence derived from the X-ray analysis, we were able in many cases to observe correspondences which confirmed both our identifications and his analysis, and to clear up ambiguities and confusions in each (Fig. 6). All in all it was possible to identify about two-thirds of all the residues in the molecule with some assurance, though some certain pairs of residues of similar shape were difficult to distinguish. We were able to summarize the results of the analysis up to this stage in the form of a model (Fig. 7) which showed the positions in space of the helical polypeptide chain segments, of the haem group, and of most of the side chains; it included, less precisely, the positions of the atoms in most of the non-helical regions and in many of the remaining side chains.

Myoglobin at 1.4Å resolution

During the past two years we have been concerned with improving the resolution of the electron density map by including virtually all the observable reflexions in the pattern, about 25,000 in number and extending to spacing of 1.4 Å; we now plot the electron density at half a million points in the molecule. It has already been pointed out that this extension of the analysis was made possible by the availability of automatic data-collecting equipment

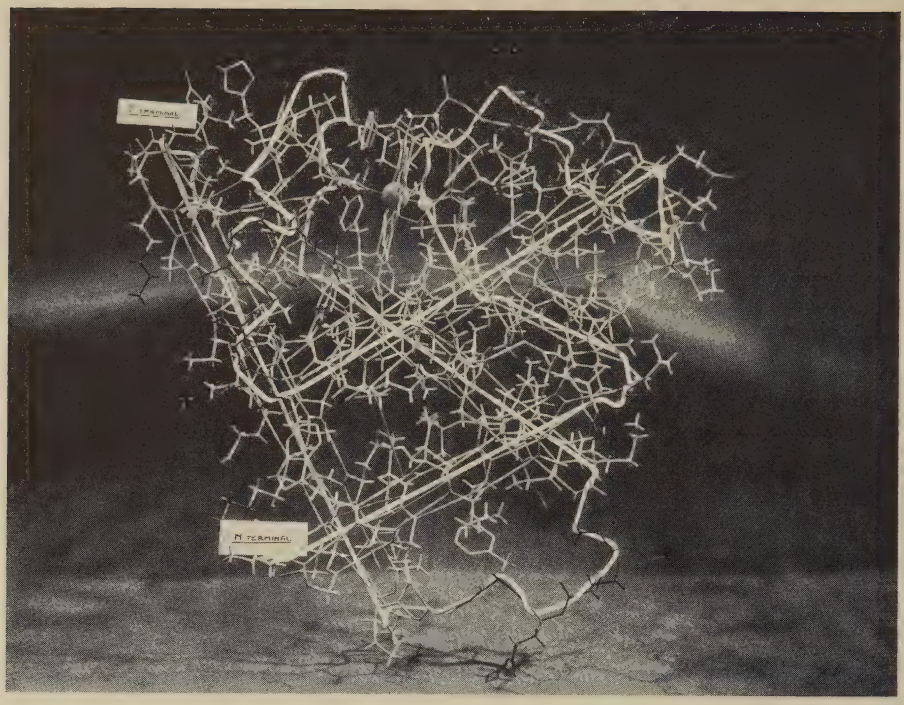

Fig. 7. Model of the myoglobin molecule, derived from the 2Å Fourier synthesis. The *white cord* follows the course of the polypeptide chain; the iron atom is indicated by a *grey sphere*, and its associated water molecule by a *white sphere*.

using proportional counters, and of still larger computers such as the IBM 7090. Even so the task would have been a very formidable one if we had continued to use the method of isomorphous replacement, involving the collection of data from a number of different isomorphous derivatives. Instead we have reverted to a more conventional method, that of successive refinement, and have abandoned the use of heavy-atom derivatives. From a study of the 2 Å Fourier synthesis we were able to assign spatial co-ordinates to about three quarters of the atoms in the molecule. Owing to the limited resolving power of this synthesis, the accuracy with which atoms could be located was a good deal less than is desirable, but this imprecision was compensated for by their number, a good deal higher in proportion to the size of the structure than is generally necessary for the success of the refinement method. This method consists in calculating the phases of all the reflexions from the co-ordinates of the atoms which have already been located; a Fourier synthesis is then computed using *observed* amplitudes and *calculated*

phases. This synthesis necessarily shows all the atoms which have been used for calculating phases, but should reveal «ghosts» of additional ones, with reduced density; it also indicates any minor errors in the positions of the atoms previously located, and if their positions are not found to coincide exactly with those assumed. One is now in a position to embark on the next cycle of refinement, using the previous set of atoms with corrected co-ordinates together with additional atoms located after the first cycle. After a few such cycles the successive Fourier syntheses should converge to a precise representation of the whole structure. We have so far carried out two cycles of refinement, including 825 atoms in the first, and 925 atoms in the second (myoglobin contains in all 1,260 atoms excluding hydrogen; in addition there are some 400 atoms of liquid and salt solution, a proportion of which are bound to fixed sites on the surface of the molecule). One or two further cycles of refinement will probably be necessary, but in the meantime the 1.4 Å Fourier synthesis based on the second cycle is very much better resolved than the 2 Å synthesis. In many cases neighbouring covalently bonded atoms are just resolved, the background between groups of atoms is much cleaner than before and, finally, many of the disturbances found in the region of the heavy atom sites in the 2 Å synthesis have disappeared. Figs. 8 (*i–iii*) will give some impression of this synthesis.

Meanwhile Dr. Edmundson has greatly advanced his study of the amino acid sequence of myoglobin; in particular he has characterized a large number of chymotryptic peptides in addition to the tryptic ones previously mentioned. Taking the results of the X-ray and chemical studies together, the situation today is that some 120 amino acid residues are known with almost complete certainty, and many of the remaining 30 with fair probability. There is little doubt that the residual ambiguities will shortly be resolved, and that the positions of all the atoms in the structure will be known with reasonable accuracy, with the exception of a few long side chains (such as lysine) which are apparently flexible and do not occupy defined positions in the crystal.

The general nature of the structure

What is the nature of the molecule which has emerged with progressively increasing clarity from successive Fourier syntheses? Some 118 out of the total of 151 amino acid residues make up 8 segments of right-handed α-helix,

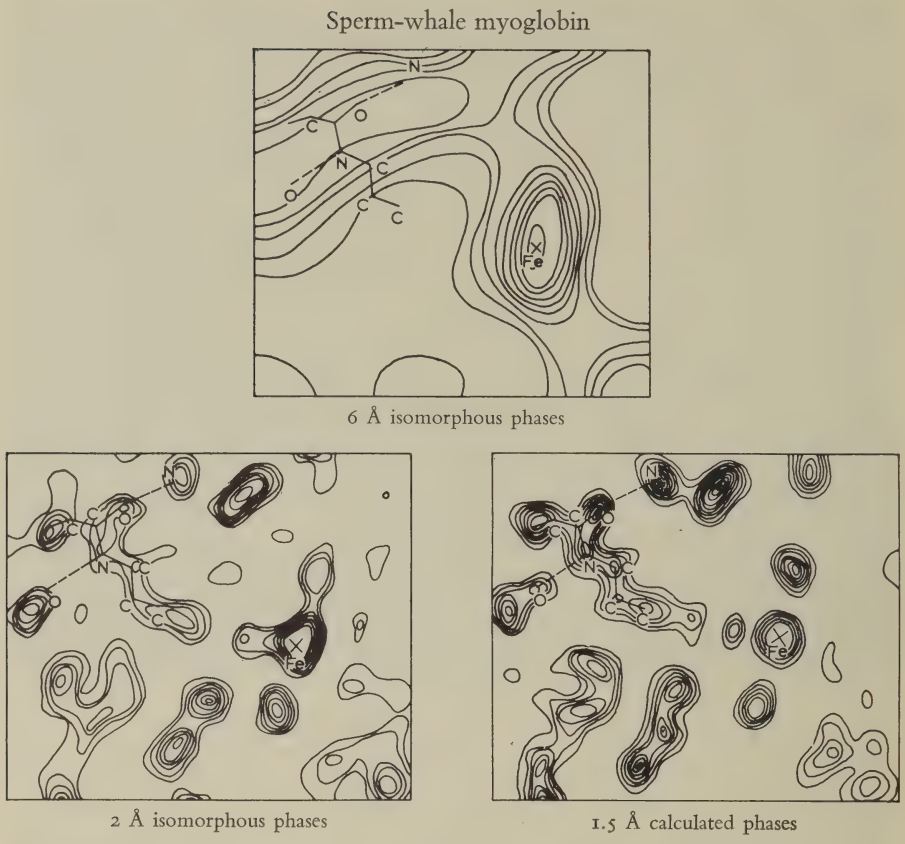

Fig. 8. Comparison between the same section through the myoglobin molecule, (*i*) at 6Å resolution, (*ii*) at 2Å resolution, (*iii*) at 1.4 Å resolution. (*Top left*) longitudinal section through a helix; (*right centre*) haem group edge-on. The atoms marked are part of the distal histidine (see text); note that several neighbouring atoms are resolved at 1.4Å.

of lengths ranging from 7 to 24 residues. These segments are joined by 2 sharp corners (containing no non-helical residues) and 5 non-helical segments (of 1 to 8 residues); there is also a non-helical tail of 5 residues at the carboxyl end of the chain. The whole is folded in a complex and unsymmetrical manner to form a flattened, roughly triangular prism with dimensions about $45 \times 35 \times 25$ Å. The whole structure is extremely compact; there is no water inside the molecule, with the probable exception of a very small number (less than 5) of single water molecules presumably trapped at the time the molecule was folded up; there are no channels through it, and the volume of internal empty space is small. The haem group is disposed almost

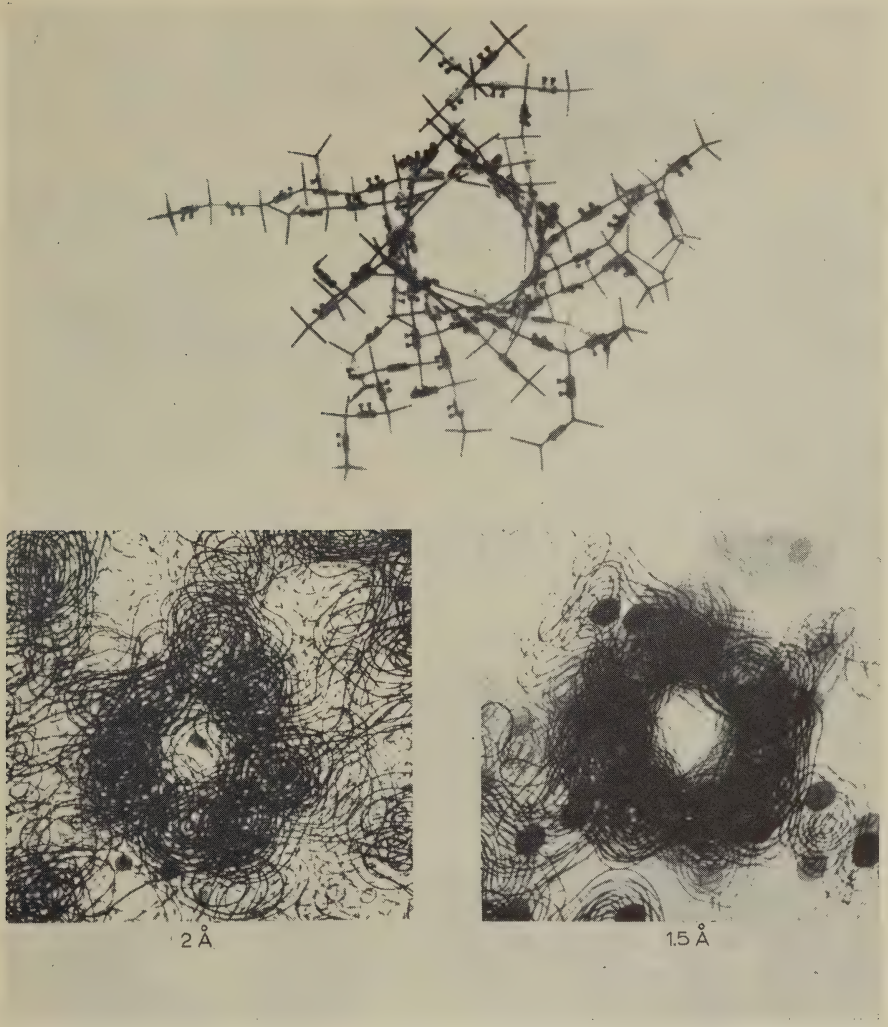

Fig. 9. End-on view of a helix at 2Å and 1.4Å resolution; (*above*) a model helix for comparison.

normally to the surface of the molecule, one of its edges (that containing the polar propionic acid groups) being at the surface and the rest buried deeply within.

Turning now to the side chains, it is found that almost all those containing polar groups are on the surface. Thus with very few exceptions all the lysine, arginine, glutamic, aspartic, histidine, serine, threonine, tyrosine, and tryptophan residues have their polar groups on the outside (the rare exceptions

appear to have some special function within the molecule, e.g. the haem-linked histidine). The interior of the molecule, on the other hand, is almost entirely made up of non-polar residues, generally close-packed and in Van der Waals' contact with their neighbours.

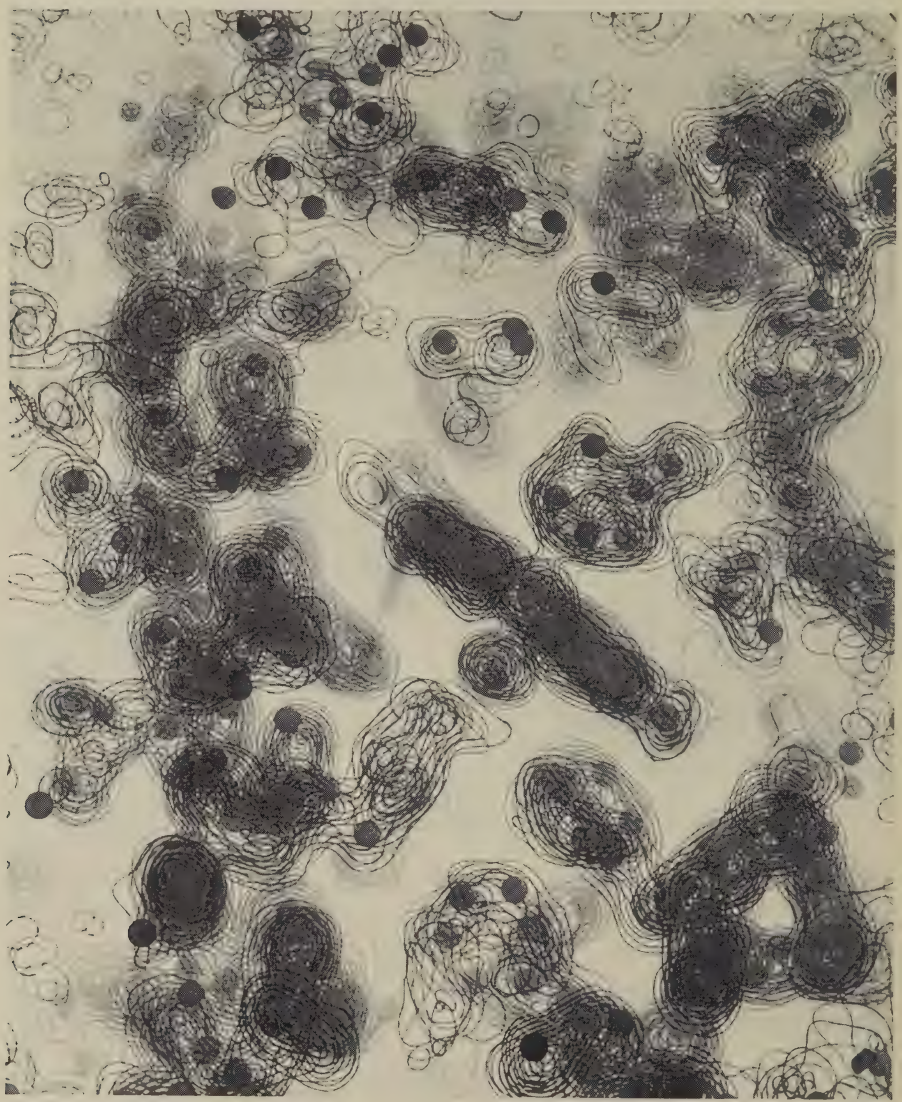

Fig. 10. Part of the 1.4Å Fourier synthesis. (*Centre*) the haem group (edge-on), showing haem-linked and distal histidines, and water molecule attached to iron atom. (*Top right*) a helix end-on. (*Bottom*) a helix seen longitudinally, together with several side chains.

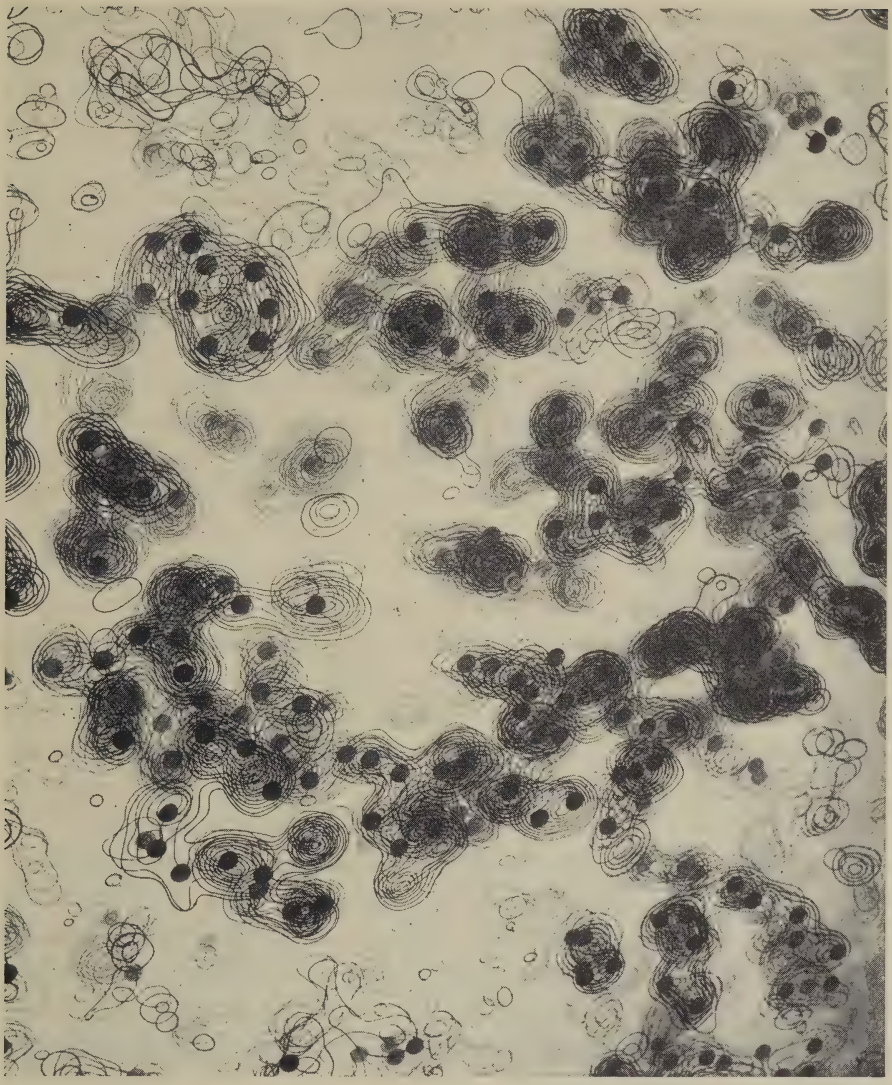

Fig. 11. Part of the 1.4Å Fourier synthesis. (*Left centre*) a tryptophan residue; (*to the left*) a liquid region between two molecules.

We may ask what forces are responsible for maintaining the integrity of the whole structure. The number of contacts between neighbouring groups in the molecule is very large, and to analyse these it has been necessary to use a large computer to calculate all the interatomic distances and to determine which of these lie within the limits corresponding to each type of bonding.

These results have not yet been studied in detail, but it is clear that by far the most important contribution comes from the Van der Waals forces between non-polar residues which make up the bulk of the interior of the molecule. It is true that there are a number of charge interactions and hydrogen bonds between neighbouring polar residues on the surface of the molecule, but one gains the strong impression that many, or even most, of these are, so to say, incidental – a polar group on the surface is quite content to bond with a water molecule or ion in the ambient solution, and only links up with a neighbouring side chain if it can do so without departing too far from its normal extended configuration. This statement should perhaps be qualified by remarking that one observes a number of polar interactions of side chains such as glutamic acid, aspartic acid, serine, and threonine with free amino groups on the last turn of helical segments; and it may be that these have some significance in determining the point at which a helix is broken and gives way to an irregular segment of chain. If so, these special interactions will be important in a wider context, as determinants of the three-dimensional structure of proteins, and might be of service in predicting the nature of the structure from a knowledge of the amino acid sequence.

The interactions of the haem group deserve special consideration. It is these which are responsible for the characteristic function of myoglobin, since an isolated haem group does not exhibit the phenomenon of reversible oxygenation. At the present time we can merely enumerate the haem group interactions; it is a task for the future to explain reversible oxygenation in terms of them. As already mentioned, the fifth co-ordination position of the iron atom is occupied by a ring nitrogen atom of a histidine residue, the so-called haem-linked histidine. On the other (distal) side of the iron atom, occupying its sixth co-ordination position, is a water molecule, as would be expected in ferrimyoglobin, the form of myoglobin used for X-ray analysis; beyond the water molecule, in a position suitable for hydrogen-bond formation, is a second histidine residue. It is noteworthy that the same arrangement of two histidines also exists in haemoglobin. For the rest the environment of the haem group is almost entirely non-polar; it is held in place by a large number of Van der Waals interactions. In haemoglobin it seems that the environment of the haem group is closely analogous, and for both proteins it is clear that a rich field of knowledge awaits exploration, for we may hope that the very extensive studies of the oxygenation reaction made during the past half-century may now be interpreted in precise structural terms.

Some implications

The oxygenation reaction of myoglobin and haemoglobin may be held to be interesting and important enough in its own right to justify the choice of these two proteins for study. In fact, as was indicated at the beginning of this lecture, the choice was originally made on different grounds, such as availability, ease of crystallization, and molecular weight. There are very many proteins which have specific functions as important, or more important; every enzyme – and many hundreds of these have been characterized – has its own specific function vital to some particular process in cell function. A number of enzymes are being studied by X-ray methods in laboratories all over the world, and in several cases the analysis is on the brink of success; a knowledge of the detailed structure of each of these will give insight into some essential biological process, by resolving the molecular architecture of the active site and permitting the same kind of interpretation of function in molecular terms as we may soon anticipate in the haem proteins. From this point of view there is no forseeable limit to the number of proteins whose structure is worth analysing, since each will have its own unique function which demands explanation in structural terms.

From another angle we may rather enquire what features are common to all proteins, and study the structure of myoglobin in its context as a typical member of this vast class of substances. Probably more experimental work has been done on proteins than on any other kind of compounds, and a huge corpus of knowledge has been built up by the organic chemist and the physical chemist. Many generalizations have been observed, but always they have been limited in scope by the fact that they could not be based on a precise molecular model. The emergence of such a model even for a single protein, such as myoglobin, makes it possible to test and to add precision to the chemist's generalizations. Already sperm whale myoglobin is being studied by biochemists in a number of laboratories with this end in view; to give only a few examples, it is being examined from the standpoint of optical rotatory power and helix content, of titration behaviour, of metal binding, chemical modification of side chains, of hydrodynamic characteristics. Such studies, and others like them, will serve to deepen our understanding of the ways in which proteins behave and of the reasons why they are uniquely capable of occupying so central a position in living organisms.

The geneticists now believe – though the point is not yet rigorously proved – that the hereditary material determines only the amino acid sequence of a

protein, not its three-dimensional structure. That is to say, the polypeptide chain, once synthesized, should be capable of folding itself up without being provided with additional information; this capacity has, in fact, recently been demonstrated by Anfinsen *in vitro* for one protein, namely ribonuclease. If the postulate is true it follows that one should be able to predict the three-dimensional structure of a protein from a knowledge of its amino acid sequence alone. Indeed, in the very long run, it should only be necessary to determine the amino acid sequence of a protein, and its three-dimensional structure could then be predicted; in my view this day will not come soon, but when it does come the X-ray crystallographers can go out of business, perhaps with a certain sense of relief, and it will also be possible to discuss the structures of many important proteins which cannot be crystallized and therefore lie outside the crystallographer's purview.

We have taken a preliminary look at the structure of myoglobin from this point of view and have to confess that the difficulties are formidable. The structure is highly irregular; the seven « corner » regions between helical segments are all different, so that generalization is impossible; the interactions between side chains are numerous and of many different types, and one cannot easily see which are crucial in determining the structure. The complexity of myoglobin is very great, yet it is probably simpler than most proteins, not only by virtue of its low molecular weight, but also in respect of its high helix content, probably much higher than that of most others. As things stand we cannot even hazard a guess as to why the helix content of myoglobin is so high, let alone see how to predict its structure in detail.

Much help with these problems may come from a comparison of myoglobin with the sub-units of haemoglobin, which Perutz has shown to resemble it very closely in spite of notable differences in amino acid sequence. By laying alongside one another the sequences of myoglobin and of the α- and β-chains of haemoglobin, and making certain plausible assumptions to explain the (fairly small) differences between their lengths, it is possible to observe homologies – points at which the same amino acid appears in a corresponding position in all three chains. The number of these homologies is surprisingly small, but presumably it is these which are responsible for the crucial interactions which determine that all three chains have the same three-dimensional arrangement (though some of the homologies may be accidents of evolutionary development). Study of homology will soon be extended by examination of other species – human myoglobin, human, horse, rabbit and human foetal haemoglobin – and of the aberrant haemoglobins

whose « mistakes » in amino acid sequence have been shown in recent years to be associated with so many hereditary diseases of the blood.

Perutz and I, with our collaborators, have already spent some time looking at these homologies, and a number of interesting facts have come to light. Yet, even in this narrow field, our studies are in their infancy; and in any case I suspect that only generalization of limited scope can be made from myoglobin and haemoglobin alone. The detailed structures of a few other proteins should soon become known, but it will be clear from many of the topics I have touched upon that we have pressing need to know the structures of very many others, for proteins are unique in combining great diversity of function and complexity of structure with a relative simplicity and uniformity of chemical composition. In determining the structures of only two proteins we have reached, not an end, but a beginning; we have merely sighted the shore of a vast continent, waiting to be explored.

The work described in this lecture has been done by many hands, and a list of those who have contributed to it, formally or informally, would be long. They come from many countries and many disciplines, and their contributions, decisive in sum, cannot be assessed in detail, and are of such varying magnitudes that any list must be invidious and incomplete. I nevertheless wish to record the following names of colleagues whose ideas and whose collaboration have been particularly important and sometimes essential.

J. M. Bennet, C. Blake, Joan Blows, M. M. Bluhm, G. Bodo, Sir Lawrence Bragg, C.-I. Brändén, D. A. G. Broad, C. L. Coulter, Ann Cullis, D. Davies, R. E. Dickerson, H. M. Dintzis, A. B. Edmundson, R. G. Hart, Ann Hartley, W. Hoppe, V. M. Ingram, L. H. Jensen, J. Kraut, R. G. Parrish, P. Pauling, M. F. Perutz, D. C. Phillips, Mary Pinkerton, Eva Rowlands, Helen Scouloudi, Violet Shore, B. Strandberg, I. F. Trotter, H. C. Watson, Joyce Wheeler, Ann Woodbridge, H. W. Wyckoff and the staff of the Mathematical Laboratory, Cambridge.

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J. C. Kendrew, Side-chain interactions in myoglobin, *Brookhaven Symposia in Biology*, 15 (1962) 216.

Biography

John Cowdery Kendrew was born on 24th March, 1917, in Oxford. His father, Wilfrid George Kendrew, was Reader in Climatology in the University of Oxford; his mother, Evelyn May Graham (Sandberg) Kendrew, was an art historian, for many years resident in Florence, Italy, where she published works on the Italian Primitives under the *nom de plume* Evelyn Sandberg Vavals.

He was educated at the Dragon School, Oxford (1923–1930) and Clifton College, Bristol (1930–1936), and went to Trinity College, Cambridge in 1936 as a Major Scholar. He graduated in Chemistry in 1939, and spent the first few months of the war doing research on reaction kinetics in the Department of Physical Chemistry at Cambridge under the supervision of Dr. E. A. Moelwyn-Hughes. He then became a member of the Air Ministry Research Establishment (later Telecommunication Research Establishment) and worked on radar. In 1940 he joined the staff of Sir Robert Watson-Watt (Scientific Adviser to the Air Ministry) and for the rest of the war was engaged in operational research at Royal Air Force headquarters, successively in Coastal Command, Middle East, and South East Asia (where he was Scientific Adviser to the Allied Air Commander-in-Chief); he held the honorary rank of Wing Commander R.A.F.

During the war years his interests became more biological, and largely owing to the influence of J. D. Bernal and L. Pauling he decided to work on the structure of proteins. He returned to Cambridge in 1946 and, in the Cavendish Laboratory, began a collaboration with Max Perutz, under the direction of Sir Lawrence Bragg. He took his Ph.D. degree in 1949 and his Sc.D. in 1962. He and Perutz were the first two members of the Medical Research Council Unit at the Cavendish Laboratory, which has now achieved separate existence as the Medical Research Council Laboratory of Molecular Biology; he was Deputy Director of the former, and is now Deputy Chairman of the latter, and Director of its Division of Structural Studies.

He became a Fellow of Peterhouse in 1947, Reader at the Davy-Faraday Laboratory of the Royal Institution in London in 1954, Fellow of the Royal

Society in 1960, and an honorary member of the American Society of Biological Chemists in 1962. Since 1960 he has been (part-time) Deputy to the Chief Scientific Adviser, Ministry of Defence. He is Founder and Editor-in-Chief of the *Journal of Molecular Biology*, and Honorary Secretary of the British Biophysical Society. In 1962, he was made Companion of the British Empire.

His research has been in the field of protein structure, and has mostly centred on the X-ray analysis of myoglobin. This project culminated in the production of a three-dimensional model of myoglobin at 6Å resolution in 1957, and an almost complete structure in 1960.

Kendrew is unmarried. His recreations are music, history of art (following his mother's footsteps particularly Italian art), and travelling in Italy.

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